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BULLETIN *of the*

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BROOKHAVEN NATIONAL LABORATORY

July 1, 1964

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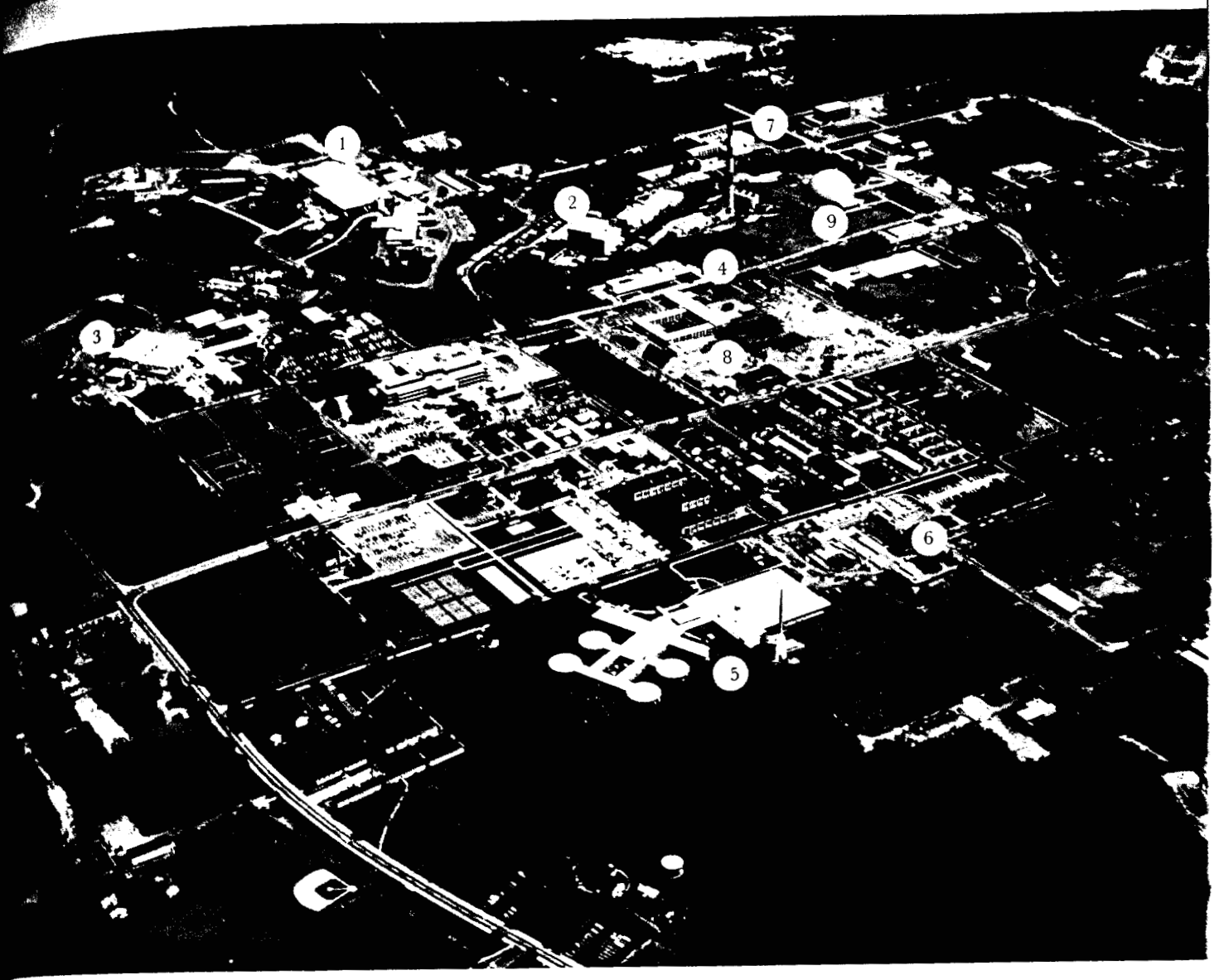
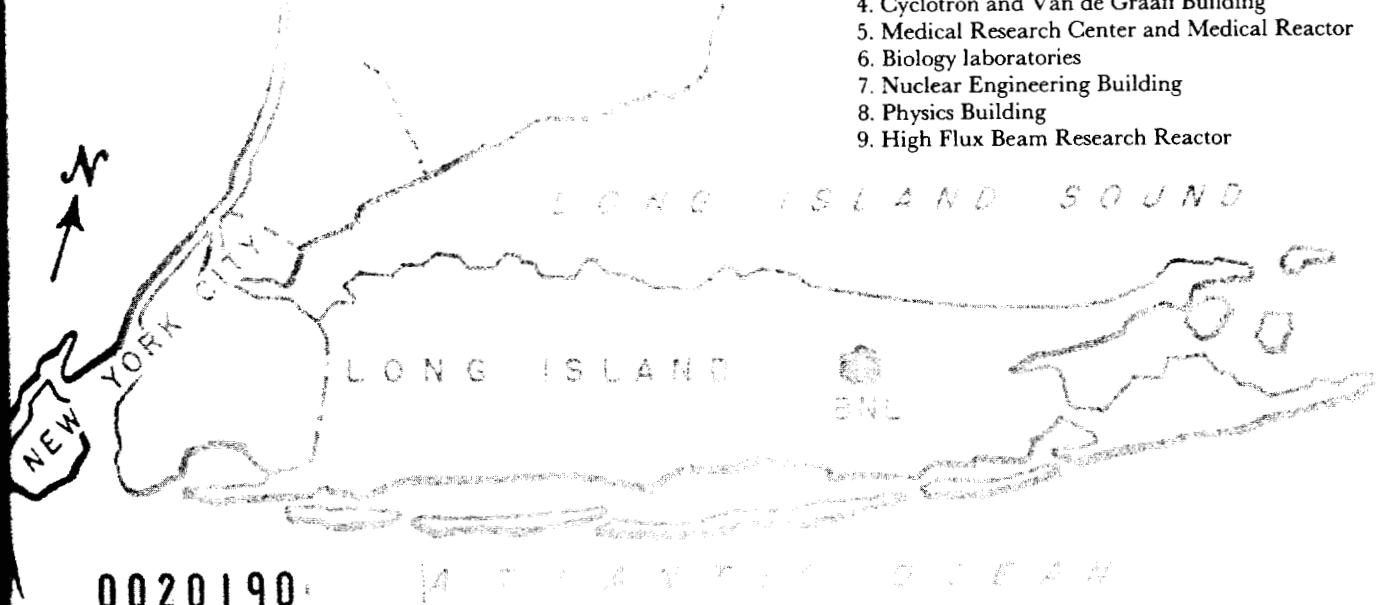


AERIAL VIEW OF THE LABORATORY

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



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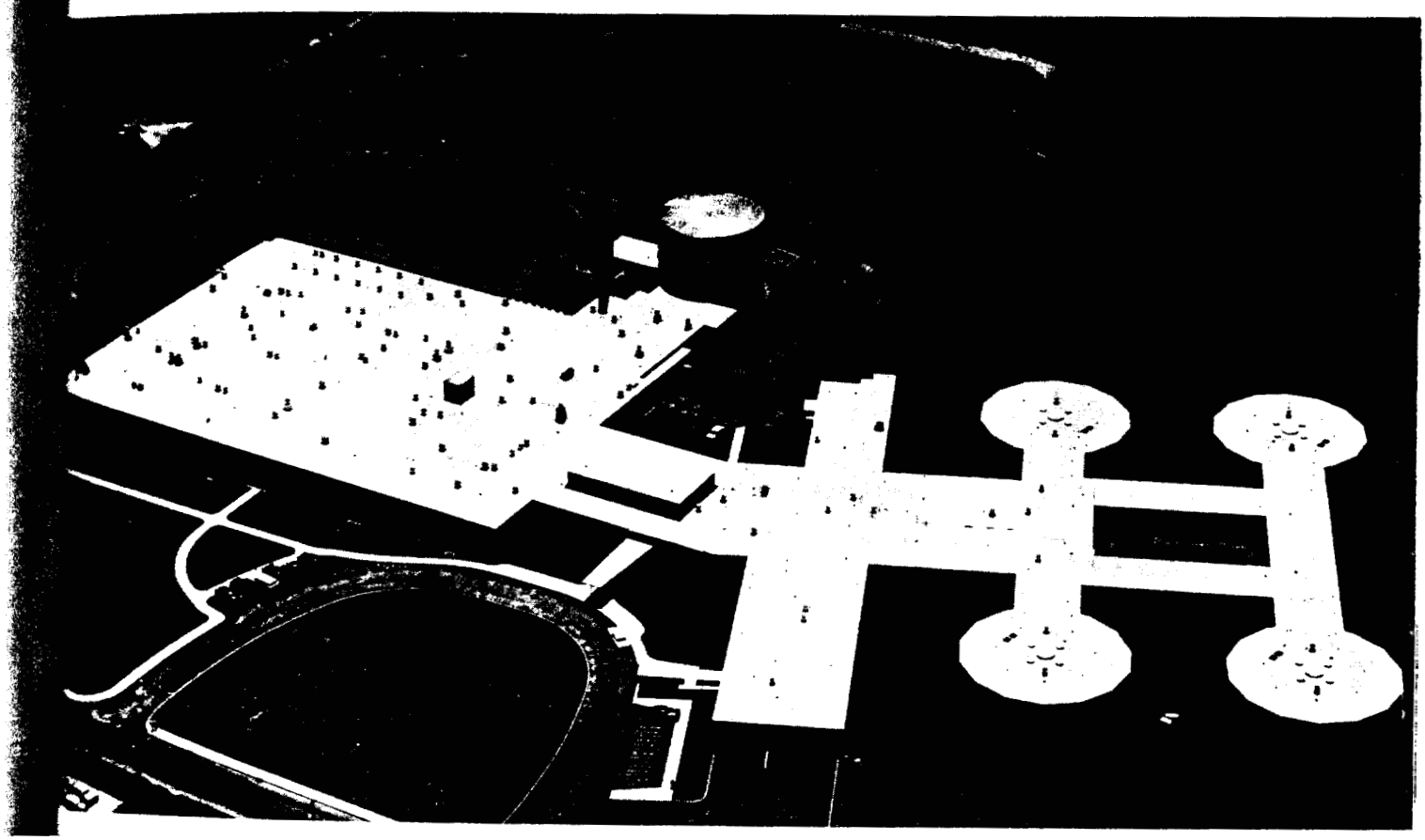


Aerial view of the Medical Department.

Laboratory Objectives and Program

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

1. To seek new knowledge in the nuclear sciences and related fields with empha on programs that require such large-scale research tools as nuclear reactors, accelerato and special laboratories which are beyond the scope of most or all individual institutio

2. To encourage appropriate use of its facilities by scientists of college, universi industrial, and other laboratories.

3. To assist the Atomic Energy Commission in the solution of specific problems 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 scie tists and engineers and otherwise to assist in the dissemination of scientific and technic knowledge.

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

These objectives, of pioneering in research fields requiring large and specialize equipment, of making the Laboratory's facilities available to visiting scientists, and furthering the education and training of young scientists, exert a profound influence o the life of the Laboratory and on its planning with respect to both staff and facilitie Because of the constantly changing work and the rotation of the staff, a maximum degre of flexibility is demanded; the continuing presence of specially skilled groups and ad quate and specialized laboratories and other facilities are required for the developmen construction, and effective utilization of advanced scientific equipments; problems housing, transportation, etc., are accentuated by the large number of scientists on tem porary assignment, by the relative remoteness of the Laboratory from centers of popul tion, and by the resort nature of the surrounding area. All these factors must be considere 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 to somewhat in excess of 20 MeV; a 3.5-MeV positive particle electrostatic 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. Also recently completed is the High Intensity Radiation Development Laboratory, which will house 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, is nearing completion.

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 400 "regular" members, 80 postdoctoral research associates with tenure limited to two years, and 400 full- and part-time visitors from other institutions. Of the last group, approximately 275 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

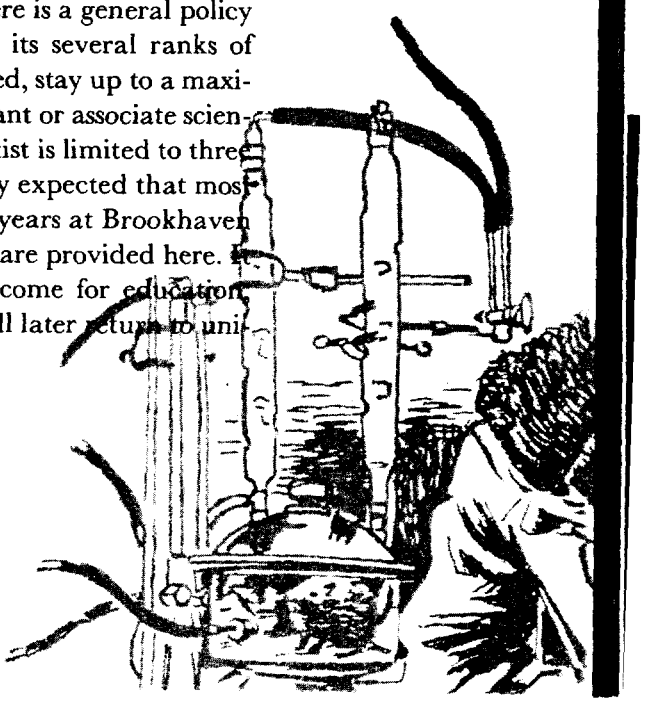
viable hemopoietic cell transfusion in the therapy of radiation injury by replacement or substitution. "Long-term" effects of radiation also are studied with respect to problems of carcinogenesis and phenomena frequently associated with aging.

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 ten 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 professorial 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.

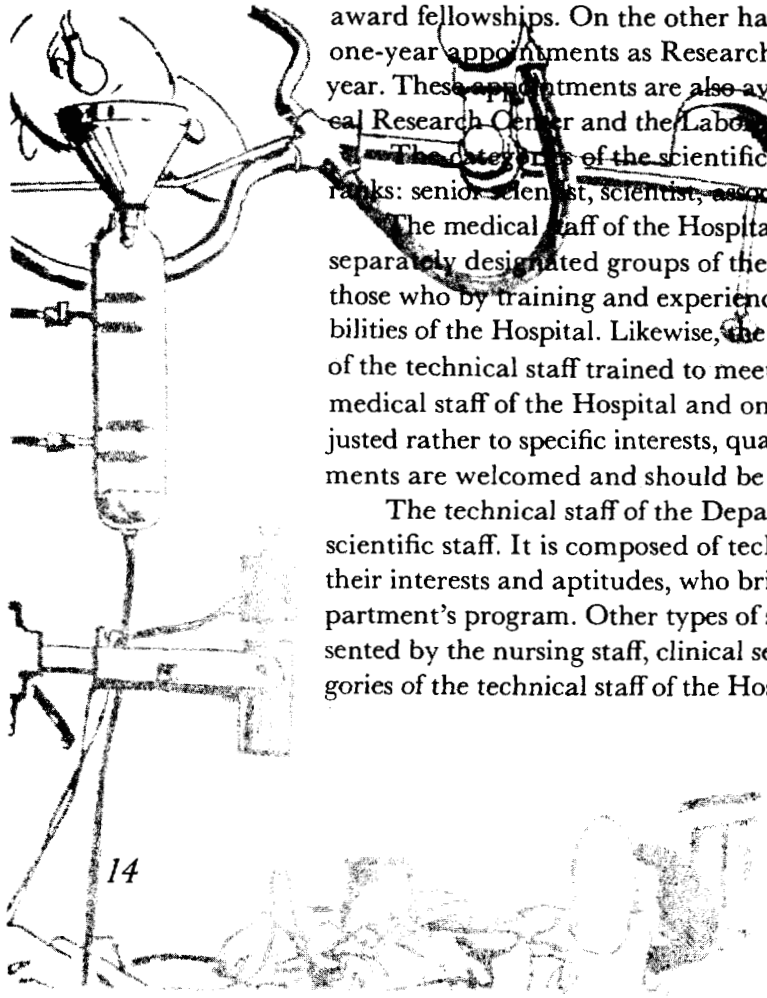
III. Research Collaborator Staff.

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.



SELECTED REPORTS ON RESEARCH ACTIVITIES DURING FISCAL 1964

The following are examples of investigative work carried on 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 infomation.

A. EFFECTS OF RADIATION

1. Biological Effectiveness of Fast Neutrons

J.L. Bateman
V.P. Bond

Over the past several years, a series of experiments has been carried out to determine, in various tissues of the mouse, the relative biological effectiveness (RBE) of fast neutrons of relatively discrete energies as compared to x or gamma radiation. The fast neutron irradiation technique employs a 3-MeV Van de Graaff accelerator with a tritium target and avoids the contaminant gamma radiation component and broad distribution of neutron energies attending radiation exposures made with reactors. The technique described permits accurate determination of effects in tissue of fast neutrons as a function of their energy level as well as comparison with the "standard" x or gamma rays.

Earlier work, noted in previous Bulletins, revealed maximum RBE values with 0.4-MeV neutrons of $\approx$4 for spleen and thymus weight loss, 5 for spermatogonia depletion, and 10 for lens opacity induction (radiation cataractogenesis). RBE values in these tissues were found to decline with rising neutron energy.

In subsequent studies, groups of mice were exposed to graded doses of (1) neutrons of 14 MeV at 1 of 3 dose rates covering a 2-decade span, or (2) neutrons of 1.80 MeV. Comparison exposures of other groups employed Co^{60} gamma rays. Determinations of spermatogonia depletion were made microscopically from testis sections and revealed an average RBE of 2.7 for the 14-MeV neutrons and 5.4 for 1.80-MeV neutrons. The 14-MeV neutron results suggested a slight dependence upon dose rate. This effect is appreciable with x or gamma rays, but could not be demonstrated previously with 0.6-MeV neutrons. The RBE values found appear to be related to the linear energy transfer (LET) associated with each type of radiation, being least for x and gamma rays, and highest for 1.80-MeV neutrons.

2. *Extracorporeal Irradiation of the Circulating Blood: Effects on Lymphocytes and Red Cells*

E.P. Cronkite
A.D. Chanana
H. Schnappauf
H. Cottier
C.R. Jansen
K.R. Rai
J.S. Robertson

The technique of irradiation of the circulating blood in an external shunt developed 3 years ago has been significantly improved during the past year by the development of better arterial venous fistulae so that flow rates of up to 1500 ml/min with a transit dose variable of from 25 to 900 rads have been obtained. In general, the system now provides for an exchange of 1 blood volume through the irradiation field every 7.5 min.

With transit doses of the order of 50 rads, a significant lymphocytopenia was attained. It has now been shown that there is a very prolonged period of time (40 or more days) during which recovery of the peripheral lymphocyte takes place.

In studies on extracorporeal irradiation combined with thoracic duct drainage, a very rapid exchange of blood lymphocytes with lymphocytic tissue lymphocytes has been shown to occur. The outflow of lymphocytes from the lymph duct primarily reflects feeding of the lymphocytic tissues by blood lymphocytes, rather than the reverse.

Studies have been initiated to compare the results of extracorporeal irradiation of leukemic and normal cows. It has been demonstrated that a lymphocytopenia can be induced in bovine chronic lymphocytic leukemia; however, the therapeutic effectiveness of this has not yet been evaluated.

With improved techniques of autotransplantation and homotransplantation of skin of cows, studies of the influence of lymphocyte depletion upon homotransplantation have been initiated. Similarly, studies are under way on the influence of lymphocytopenia produced by extracorporeal irradiation upon the capability of inducing a primary immune response by tetanus toxoid. Major efforts will be made to adapt this technique to the study of appropriate clinical material such as chronic lymphocytic leukemia, acute leukemias, and autoimmune diseases.

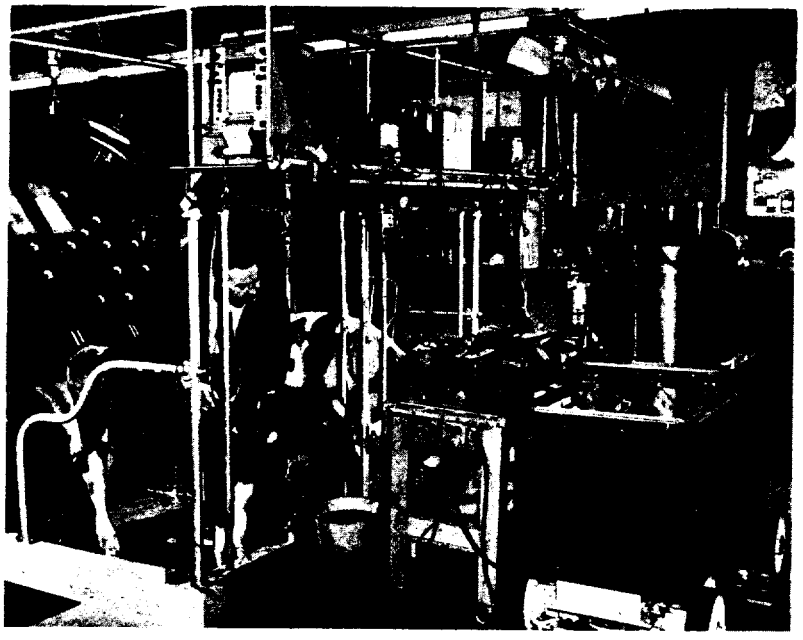


Figure 1. Extracorporeal irradiation of the blood in the new large animal facility.

3. *Effects of High Energy Particles on Mammals*

V.P. Bond
J.E. Jesseph
J.S. Robertson
G.M. Tisljar-Lentulis
W.H. Moore, Jr.
(Accelerator Department)

With the cooperation of the staff of the Cosmotron, studies of the effects of protons in the 2.0 to 3.0-BeV range are continuing, and studies with π^- mesons have been initiated.

In the past year, a considerable effort has been devoted to the numerous factors involved in delivering completely uniform whole-body proton radiation to small animals. Such uniformity is critical to meaningful studies of lethality. It was found that the LD_{50} for mice for 2.2-BeV protons is $(8.25 \pm 0.12) \times 10^{10}$ particles, provided that the protons are uniformly distributed over the cross-sectional area of the animal's body. Departures from this "flat" field of as little as 15% have the effect of lowering the lethal dose by nearly one-half. This effect is thought to be due to a preferential irradiation of radiosensitive organs and tissues, since the irradiations are given along the longitudinal body axis. From these studies, it is hoped to establish values for the RBE of protons in this energy range, and methods are being designed for study of LET and of the nature and contribution to dose of the secondary particles resulting from nuclear interactions of the primary (proton) beam.

Based on theoretical considerations which attribute a high depth-to-surface dose ratio to π^- mesons, an investigation of such particles with respect to their applicability to certain problems in radiobiology and radiation therapy is in progress. When a beam of π^- mesons is stopped in an absorber, many of the particles will be captured at the end of their paths by one of the absorber nuclei, and energy equivalent to the meson's rest mass ($\approx$140 MeV) will be set free. A low-intensity beam from the Cosmotron was used, and positive and negative particles of low momentum were studied with a tissue-equivalent ionization chamber and a counter telescope. The momenta of the particles were chosen to correspond to a range of 6 in. in wet tissue. The identification of the π^- mesons among other particles of the same charge and momentum was done by means of coincidence-anticoincidence techniques, which make it possible to measure the effects of interest even in the presence of a heavy background of undesired particles. Absorption curves indicating the number of particles with increasing penetration into an approximately tissue-equivalent absorber as well as range curves of the beam were determined. A multichannel analyzer supplied information on the amplitude distribution of the electronic pulses that result from the particles' energy losses in plastic scintillation counters. The change of this distribution with increasing absorber depth indicates the increase of energy transfer from the particles to the absorber material and makes it possible to observe the meson capture events with their particularly high energy release. After experience has been gained with the equipment now in use, quantitative dose mea-

surements will be carried out. Biological effects will be studied when π -meson beams of high intensity and purity become available.

4. Antibody Responses in Thymectomized Mice

M.W. Hess
H. Cottier
R.D. Stoner

Renewed interest has recently arisen in the importance of the thymus in immune mechanisms and its possible role in the development of immunologic competence. The present study deals with the effect of thymectomy in newborn mice upon the development of their ability to produce antibody as adult animals.

The thymus was surgically removed from 1 to 8 days after birth. All animals were deeply anesthetized with ether. A median incision was made through the skin and sternum. The opening in the chest was expanded with a reverse-action wire retractor to expose the thymus as shown in Figure 2. Both lobes of the thymus were removed by blunt dissection with a wire loop and iris forceps. (Figure 3 shows the appearance of the chest cavity after removal.) The thymectomized and the nonoperated littermate control animals were given a primary immunization with tetanus toxoid when 4 wk of age. A booster injection of toxoid was given 3 wk later to elicit secondary antitoxin response. With these time intervals, thymectomized mice showed only slightly

Figure 2. Opening in chest of mouse to expose thymus.

Figure 3. Chest cavity of mouse after removal of both lobes of thymus.

impaired primary antibody response but severely repressed secondary response as compared to the littermate controls. These data indicate that postnatal thymectomy does not abolish the ability to respond later to antigenic stimulation at the age of 4 wk. Subsequent studies are concerned with the hypothesis of a migration of stem cells from the thymus to other lymphoid organs. There is no indication in the strain of mice used in these studies of a marked peripheralization of thymic lymphocytes prior to birth.

5. *Enhanced Antibody Responses Elicited With Tetanus Toxoid Complexed With Antibody*

R.D. Stoner
G. Terres
M.W. Hess

Mice were immunized with either a single injection of tetanus toxoid only or a single injection of toxoid complexed with mouse antitoxin (antibody to toxoid). The complexes were prepared in toxoid-antibody ratios of antibody excess, equivalence, and toxoid excess. Primary antibody responses were detected earlier along with greatly increased amounts of antibody in mice immunized with the complexed toxoid as compared with responses obtained in mice immunized with the same amounts of antigen only. Enhanced antibody responses were also obtained in irradiated mice (400 rads) when the various complexes were injected one day after irradiation. The greatest degree of enhancement of primary antibody responses in normal (Figure 4) and irradiated mice occurred when the complexes were prepared in ratios of equivalence and antigen excess. The demonstration of the enhancement phenomenon provides conclusive evidence that the antibody portion of the complex need not be of foreign origin in order to elicit

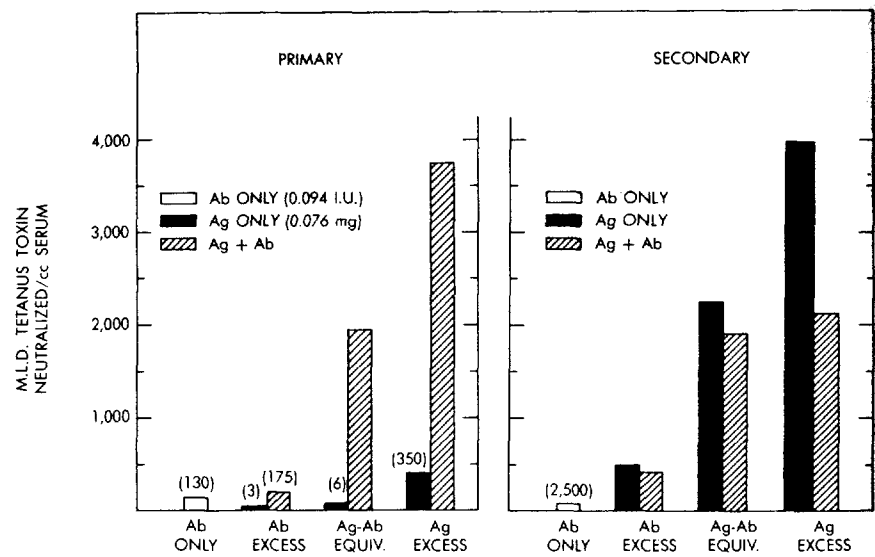


Figure 4. Comparison of primary and secondary tetanus antitoxin responses in nonirradiated mice elicited either by toxoid (antigen) or with the same amounts of toxoid complexed with mouse antitoxin. (Ag = antigen; Ab = antibody.)

enhanced antibody formation. Secondary antibody responses were slightly depressed when booster injections of the various complexes were used to elicit booster responses. Corresponding depression of secondary responses was observed in irradiated mice (400 rads) given booster injections of the various complexes. Complexes of toxoid and mouse antibody are more efficient in eliciting primary responses, whereas the same amount of antigen only is more efficient in eliciting booster responses.

6. Lens Opacification With Ionizing Radiations

J.L. Bateman
V.P. Bond
H.A. Johnson

The development of opacities in the lens of the human eye after exposure to ionizing radiation has become of increasing concern in accidental and occupational exposure and in radiotherapy. Experimental interest in the mechanism by which these opacities develop has focused partly on the high RBE of radiations such as fast neutrons in causing lens opacities when compared to radiation by x or gamma rays. Recent investigations of the latter have been made at this Laboratory, employing specialized examination techniques. Study of the lens in experimental animals has generally involved periodic visual examination with either an ophthalmoscope or a slit-lamp microscope. In the current studies a slit-lamp microscope was employed in conjunction with a mechanical mouse restrainer as shown in Figure 5. Anterior and posterior lens opacities, found to differ significantly in appearance and growth, were scored separately, rather than "lumped" as has been the usual practice elsewhere.

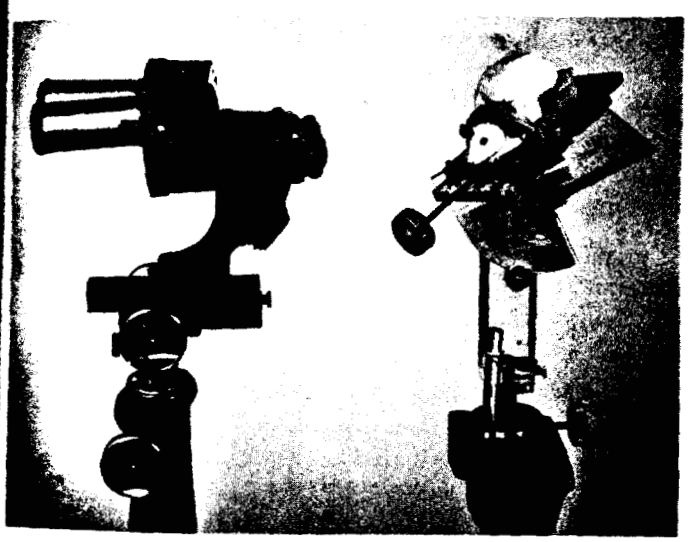


Figure 5. Lateral view of the facility employed in examination of the mouse, showing the approximate position for study of the right eye. The light source, usually positioned almost parallel and adjacent to the microscope, has been omitted for clarity.

In the present investigations, lens opacification was studied as a function of time following the exposure of 16-wk-old mice to low and divided doses of 250-kVp x rays or to neutrons at energies of either 0.43 or 1.80 MeV. (The monoenergetic fast neutron technique, utilizing a 3-MeV Van de Graaff generator in conjunction with a tritium target, was employed for these studies in the same manner described in previous Bulletins for earlier studies of other cell systems in the mouse.) The data have revealed to date an increase in lens opacification for all three radiations, with either time or dose, even for values of the latter as low as 1 rad of neutrons. An RBE of ≈ 10 has been obtained for lens opacification by neutrons of either energy tested, when compared to x rays. Posterior opacities have been found to appear earlier and to progress more rapidly than those located at the anterior pole of the lens.

Studies on fine structure alterations during formation of radiation-induced cataracts, using conventional and electron microscopy, are under way.

7. Action of Radiation on Bacteria in the Presence of Oxygen

A.A. van Soestbergen

The objective in these studies is the definition of factors influencing radiation effects on bacteria in the presence of high oxygen concentrations, particularly the type of radiation, the optimal environmental circumstances, and the physiologic conditions.

An experimental setup for x irradiation of bacteria under carefully controlled oxygen concentrations has recently been completed. Methods to irradiate bacteria with densely ionizing particles (Van de Graaff generator and cyclotron) and neutrons of different energies (obtained from the Medical Research Reactor and the Van de Graaff generator) are under study and will shortly be put into practice.

It has already been shown, with *Pseudomonas aeruginosa* used as the test subject, that the relationship between the percentage of bacteria surviving an x-ray dose and the logarithm of the oxygen concentration can be adequately described by a cumulative normal curve. Statistical treatment of the data by probit analysis has thus become feasible.

In a study of postirradiation treatment in modifying the effect of x irradiation, it was demonstrated that acriflavin does not exert an effect on *Pseudomonas aeruginosa* as has been reported in the literature for *E. coli* B/S. Therefore the effect of comparable treatment on *E. coli* B/S is also under investigation. As a preliminary to studies involving the effect of incorporation of DNA base-analogues on the radiation sensitivity of bacteria, the effect of glucose starvation was measured. The

radiation sensitivity of the bacterial population was increased to an appreciable extent. This increase in sensitivity appeared to be more of a quantitative than of a qualitative nature.

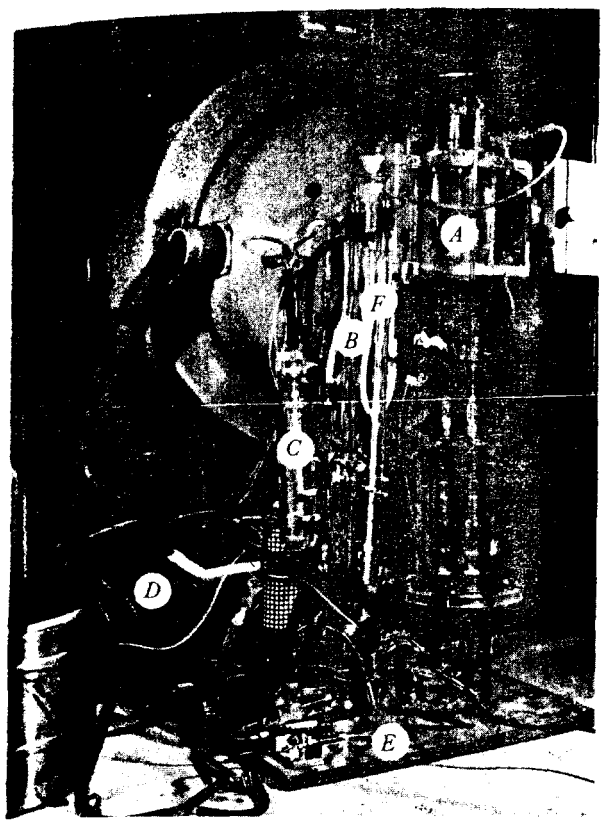


Figure 6. X irradiation of bacteria under controlled oxygen concentrations. The two vessels containing the bacterial suspensions (*A*) are inside a water bath which is secured to the x-ray machine. Gas velocity is measured by means of a soap bubble in the burets (*B*). Next to the burets is a Hersch cell (*C*). The current produced by this cell is recorded by a recorder (*D*). Gas of known oxygen content is led into the vessels through copper or glass tubing (*E*). A Victoreen ionization chamber (*F*) is attached to the water bath.

8. Protective Effect of Tail-Shielding in Irradiated Mice

C.V. Robinson
S.L. Commerford
J.L. Bateman

It has been found in a preliminary series of experiments that shielding the tails of mice when they are exposed to an x-ray dose of 600 to 800 r provides some protective effect. Mice with tails shielded (group *a*) and with tails unshielded (group *b*) were exposed to the same trunk doses. On the sixth day post irradiation, the spleens of group *a* mice weighed about twice as much and had about 20 times the iodine-labeled deoxyuridine uptake (activity per spleen 6 hr after injection) compared to the group *b* mice. Similar but smaller effects were found when half the tail was shielded.

The above results imply the presence of cells in the mouse tail capable of recolonizing irradiated hemopoietic tissue. Histological

examination of sections of tails of animals from the above experiments fixed at the time of sacrifice (6 days post irradiation) and of normal tails from mice of the same strain (Hale-Stoner) shows the presence of significant amounts of hemopoietic tissue in shielded and normal tails. This finding and the protective effect are at variance with a widely held view, expressed in the literature, that the mouse tail does not contribute significantly to hemopoiesis.

It is of interest to compare the protective effect of tail-shielding with that obtained by injection of isologous or homologous bone marrow as studied by a number of other workers, notably J.E. Till and E.A. McCulloch, and G. Cudkowicz. They have shown a linear relationship between spleen regeneration and number of cells injected. The tail-shielding method of transplantation is more physiological and not subject to interference by the immune mechanism. On the other hand, the time of transplantation and the number of cells transplanted are not subject to direct control. Experiments are in progress to clarify details of the mechanism of transplantation from the shielded tail as to time of migration and cell type or types involved, and to correlate quantitatively the numbers of viable hemopoietic cells in the shielded tail with spleen regeneration.

If shielded tails can serve as biological dosimeters, and thus as a basis for determining RBE's of radiations of different qualities, this method would have the advantage over the whole-body lethality method that the tail is a more homogeneous cellular system, contained in a smaller and more convenient volume.

9. Radiation Life Shortening

H.A. Johnson
E.P. Cronkite

Relationships between parameters of aging and those of radiation life shortening have been studied by setting up simulated experiments on the IBM 7094 computer. By using functions obtained from experimental data for aging and radiation life shortening, it has been possible to generate hypothetical survival curves for mice irradiated at various ages. These studies have shown that if aging and radiation life shortening have certain mechanisms in common, their effects will not be additive, but sensitivity to the life-shortening effect of irradiation must decrease with advancing age.

As a part of this study, a large group of mice were injected with tritiated thymidine in order to define more accurately the risks involved in the clinical use of this compound. The results are being compared with those from an untreated group and from a group that received whole-body gamma radiation. In addition to its clinical im-

portance, this experiment provides a unique opportunity to observe life spans, mortality rates, and tumor incidences in animals receiving chronic radiation directed almost exclusively at cell nuclei.

10. In Vivo *Measurement of Radionuclides in Man: Kinetic Studies and Body-Burden Measurement*

S.H. Cohn
J.S. Robertson
S. Bozzo
C. Constantinides
J.E. Jesseph
D. Huene
T. Teree
R. Love
E. Gusmano

The sensitivity, precision, and accuracy of the whole-body counter permit the measurement of the long-term biological turnover of various elements in man with low levels of tracer. Turnover rates are being obtained to aid in assessing the hazard to man from fission products and other radionuclides. The turnover rates of Sr^{85}, Cs^{137}, Zn^{65}, and Sc^{46} in patients are measured for periods of 1 yr or longer, and mathematical models to represent the data are developed. From these models, extrapolations can be made for relatively long periods. With this baseline information it is possible to evaluate the effects of various agents on the biological turnover rates. Sr^{85} turnover can be described equally well over a 1-yr period by a power function or by a series of exponential functions. Cs^{137} and Sc^{46} retention data can be described adequately by single exponential functions with half-lives of 80 and 1500 days, respectively, while Zn^{65} is best described by a 2-compartment model with half-times of 12 and 335 days.

The whole-body counter also permits the rapid identification and quantification of body burdens of gamma emitters in large populations. A FORTRAN II program for the IBM 7094 was developed for reduction of complex pulse-height data to quantify the levels of Cs^{137}, Zn^{65}, and K^{40} in 600 Brookhaven employees. The program (referred to as GAMSTRIP) performs a nonlinear least-squares fit of a Gaussian function to the total absorption peak. The associated Compton contribution required in the program is computed by interpolation from an experimentally derived matrix of seven monoenergetic gamma rays in a phantom.

Compartmental models of calcium and strontium metabolism were developed by analogue and digital (IBM 7094) computer analysis of the Sr^{85} and Ca^{47} data from short-term kinetic tracer studies. Kinetic studies have been carried out on patients with normal skeletal metabolism and patients with partial parathyroid deficiency. These kinetic studies utilizing Sr^{85} and Ca^{47} were extended to permit comparisons of the biological turnover following chronic administration with that following acute parenteral administration. A current chronic feeding study, using Sr^{85} in four patients, allows a more complete labeling of the "stable" bone compartments with long time constants. By this means it should be possible to derive the function describing the rate of bone resorption and long-term exchange from "stable" bone compartments.

A new technique was developed to measure Sr^{85} and Ca^{47} turnover in a bone compartment by external scanning with a collimated detector. Data from a study on several patients with this technique show differing uptakes in contralateral homologous bones as a function of the amount of use of a limb.

The kinetic studies of calcium and strontium turnover in rats have been continued and supplemented by the use of a newly developed neutron activation technique for the analysis of stable strontium in plasma and tissues. This analytical technique has been applied in a study of the effects of stable calcium and strontium feeding on the turnover of Sr^{85} in blood and other tissues. In kinetic studies on infants and children, stable strontium tracer will be used, since the use of radioisotopes is not desirable. Other experiments are in progress to measure the turnover rate in rats of the mineral phase of homogenous and autogenous sterilized orthotopic bone grafts utilizing Sr^{85} and Ca^{47} as tracers.

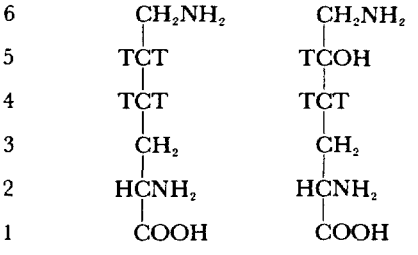
Studies on the *in vivo* measurement of Thorotrast have continued with emphasis on the gamma-ray spectrometric analysis. Because of the complexity of the decay chain of Th^{232}, the interpretation of the spectral data is carried out with the IBM 7094 computer. The levels of gamma-emitting daughters of Th^{232} (Tl^{208} and Ac^{228}) in various organs were determined by comparison of the Thorotrast patient's spectrum with that of a phantom containing a calibrated solution of $Th(NO_3)_4$ at secular equilibrium. Long-term studies are also in progress to measure the turnover of injected Thorotrast in rats.

B. STUDIES ON BIOCHEMICAL STRUCTURES AND FUNCTIONS

1. The Biosynthesis of Hydroxylysine

E.A. Popenoe
R.B. Aronson
D.D. Van Slyke

The 5-hydroxylysine of collagen can arise only from lysine, a fact established in earlier experiments in this Laboratory. During a further study of collagen biosynthesis in polyvinyl sponge biopsy tissue taken from rats, it was found that only one of the four labeled hydrogen atoms of lysine-4,5-H^3 was lost during conversion to hydroxylysine.



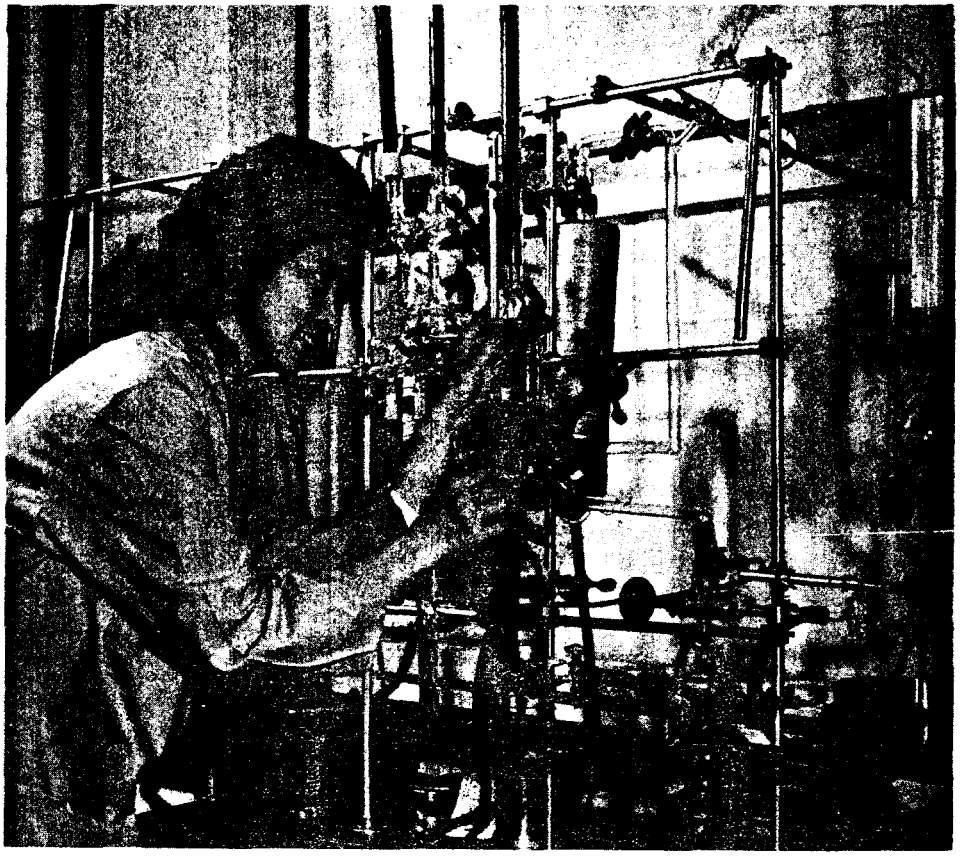


Figure 7. In the study of hydroxylysine biosynthesis, tritium from labeled samples is transferred in this apparatus to tubes for counting.

Although this result excluded 4-5 unsaturated lysine and 5-keto lysine as possible intermediates in the biosynthesis of hydroxylysine, it left at least two possible mechanisms open: the conversion might take place by direct oxidation of carbon atom number 5, or by the production of a double bond between carbon atoms 5 and 6, followed by the addition of the elements of water to form hydroxylysine.

To explore the mechanism of conversion further, experiments of two types are under way. First, collagen synthesis is being carried out in the presence of tritium-labeled water, and second, lysine labeled with tritium on carbon number 6 has been prepared for use as a tracer. If the tritium label in lysine-6-H^3 remains intact during the formation of hydroxylysine and if no tritium is incorporated in the tritium water experiments, then the direct oxidation mechanism will seem most likely. On the other hand, if a part of the tritium of lysine-6-H^3 is lost and tritium from labeled water is incorporated, formation of an intermediate 5-6 double bond will be indicated.

B. STUDIES ON BIOCHEMICAL STRUCTURES AND FUNCTIONS

2. *Cause of the Nonreactivity of Hydroxylysine in Collagen*

R.B. Aronson
C. Franzblau
D.D. Van Slyke

Collagen forms ≈30% of the protein of the mammalian body and serves to hold together the structural form of the organs. Hydroxylysine is an amino acid that occurs only in collagen and presumably gives the latter some of its unique properties. Hydroxylysine has the structure $NH_2 \cdot CH_2 \cdot CH(OH) \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$. If it is bound in the collagen molecule by the usual peptide linkage of its carboxyl and adjacent α-amino group, the hydroxyamino group $NH_2 \cdot CH_2 \cdot CH(OH) \cdot$ shown at the left of the above chain should be free and capable of entering into chemical reactions unless it either (1) is made inaccessible by steric hindrance due to its geometrical place in the protein molecule, or (2) is made nonreactive by chemical combination of the NH_2 or OH group. Reaction with periodate oxidizes the group $NH_2 \cdot CH_2 \cdot CH(OH) \cdot R$ to $NH_3 + CH_2O + OCH \cdot R$. With free hydroxylysine the reaction is complete in a few minutes. When the periodate reaction is applied to gelatin made from the collagen of bone, however, only 60% of the hydroxylysine is found to be destroyed, 40% being completely nonreactive. To determine whether the nonreactivity is due to steric hindrance, the gelatin has been split into small peptides by digestion with collagenase, and the periodate reaction has been applied to the digest. If the nonreactivity were due to steric hindrance, the enzymatic hydrolysis to peptides should remove the hindrance. However, the digest showed, as did the intact gelatin, 40% of the hydroxylysine to be nonreactive. The results indicate that either the NH_2 or the OH group of the hydroxylysine in collagen is probably bound in chemical linkage. Such combination could contribute cross-links essential to the structure of the collagen molecule. Work is planned to identify the chemical nature of the linkage.

3. *Ribonucleic Acid Structure in Normal and Tumor-Virus Infected Cells*

N. Delihas

Little is known about the primary structure of the various classes of ribonucleic acids (RNA's) in the normal and neoplastic states. Information on RNA nucleotide sequences in tumors may be important in understanding the development of cancers, since it has been shown that RNA can both induce tumor formation and, in some instances, inhibit the growth of tumor cells.

Studies have been made of the structure of RNA's extracted from normal chick embryo chorioallantoic membranes and those infected with the Rous sarcoma virus. An analysis of the structure of 30 S and 18 S RNA's from normal chorioallantoic membrane cells has shown that there are distinct differences in the nucleotide sequences of these two RNA's. This was first observed when differences were found in the pattern of enzymatic digestion of the two RNA's (Figure 8).

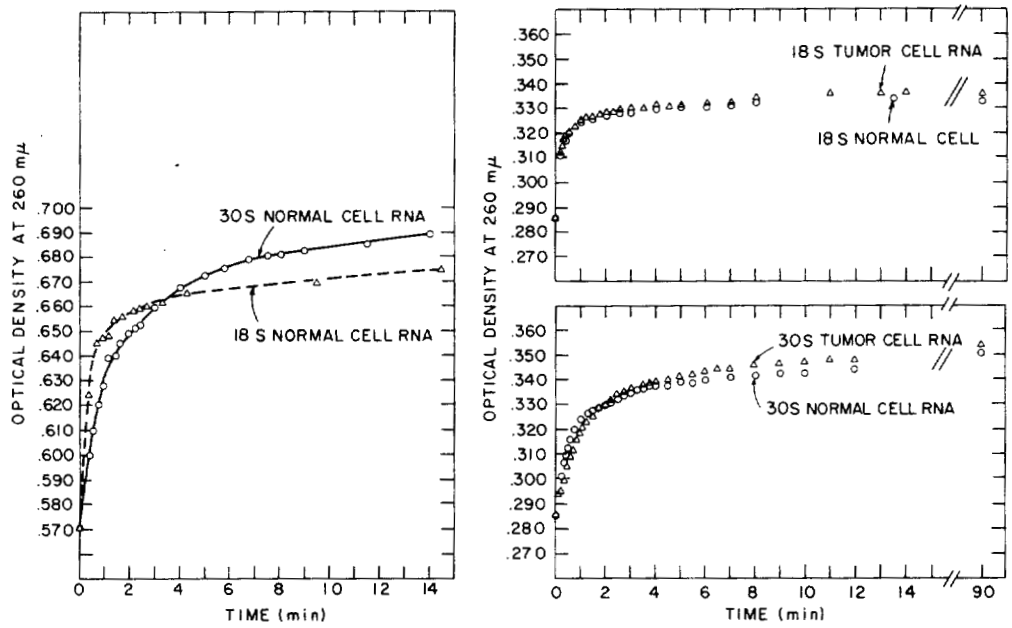
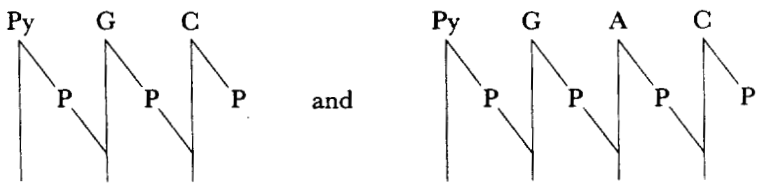


Figure 8. Pancreatic ribonuclease digestion of normal and tumor tissue RNA.

Following DEAE-Sephadex ion exchange chromatography and analysis of the oligonucleotide products of ribonuclease digestion, it was found that 30 S RNA appears to have a much greater abundance than 18 S RNA of the following proposed sequences of nucleotides:



where

Py = pyrimidine nucleoside,
P = phosphate linkages,
G = guanosine,
C = cytidine,
A = adenosine.

B. STUDIES ON BIOCHEMICAL STRUCTURES AND FUNCTIONS

After infection of embryos with the Rous sarcoma virus, identical differences in 30 S and 18 S tumor RNA's were found; however, no differences between normal and tumor RNA were detected either in the 30 S or 18 S RNA's. The patterns of ribonuclease digestion were similar, and chromatograms of the digests revealed no significant differences between normal and tumor extracts.

A comparison of oligonucleotide digestion products of extracts from highly purified nuclei and from the cytoplasm of normal membrane cells has indicated a similarity in nucleotide sequence arrangement (Figure 9). This finding supports recent evidence from other laboratories that some RNA's of normal cell nuclei are similar to cytoplasmic ribosomal RNA's. Additional studies with ribosomal RNA's extracted from the cytoplasm and RNA's from purified cell nuclei of Rous sarcoma cells indicated very close similarity of clusters of nucleotides between normal and tumor tissue, which confirmed the findings with 30 S and 18 S total cell RNA.

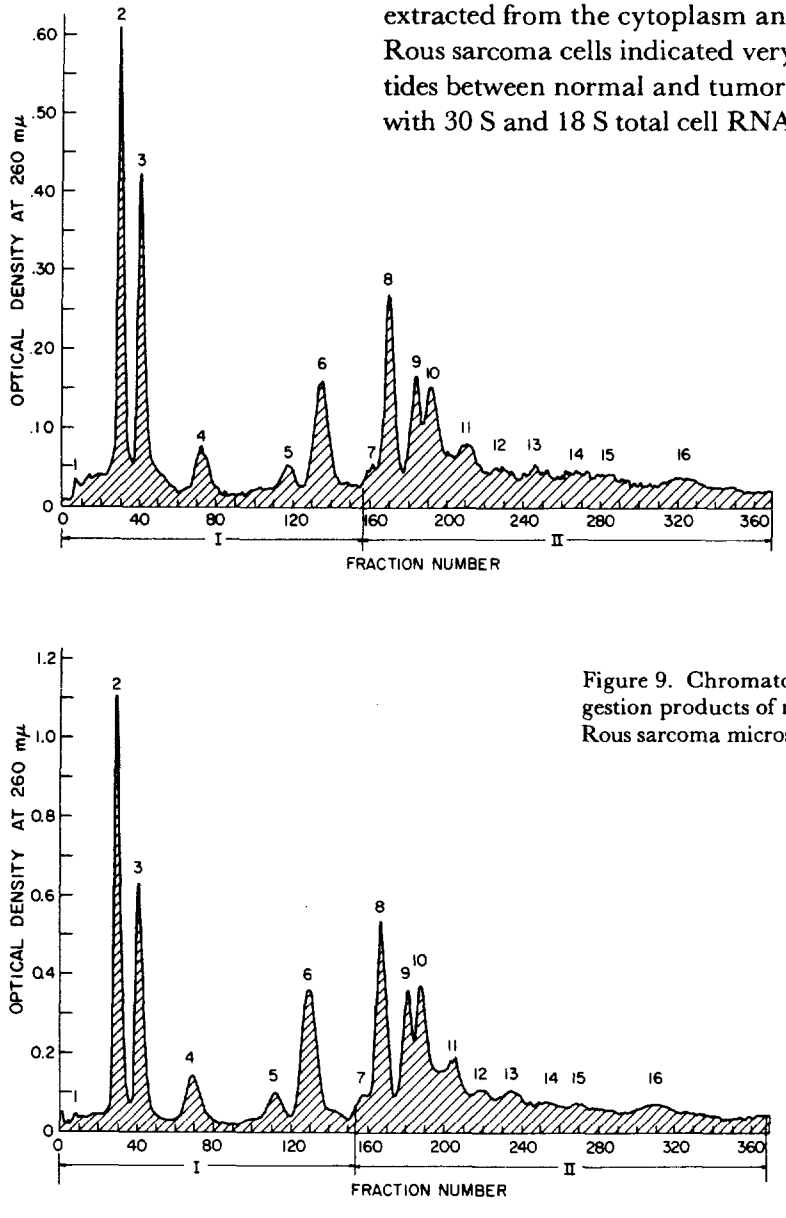


Figure 9. Chromatographic profiles of ribonuclease digestion products of normal microsomal RNA (top) and Rous sarcoma microsomal RNA (bottom).

These results with Rous sarcoma tissue suggest that in this particular tumor ribosomal RNA's may be largely unaltered in structure and that these RNA's may be functioning in a fashion similar to normal cells in the synthesis of cell proteins.

4. Instrumental Methods in Radioactivation Analysis

D.C. Borg

Instrumental procedures used in radioactivation analysis, although generally less sensitive than radiochemical separations, usually are nondestructive, faster, and easier. Furthermore, since instrumental techniques often can provide multielement analyses of single samples, they are particularly advantageous in the analysis of biological materials characteristically containing many elements that become activated upon irradiation by neutrons (or charged particles).

Previous work at BNL developed general methods for applying multiple coincidence spectrometry with "counter-telescopes" and selective irradiations with filtered reactor neutrons to radioactivation analysis of medical and biological material. The coincidence spectrometer itself also was useful for measuring and resolving samples of mixed gamma-emitting radioisotopes from tracer studies.

An improved coincidence spectrometer is now being assembled containing a larger NaI crystal with improved spectral resolution for gamma rays, a redesigned beta-ray counter-telescope, and a coincidence control unit capable of speeding up the resolving time by a factor of 10. Provision has been made to utilize the spectrometer with a 4096-channel, 2-parameter (X-Y) multichannel analyzer in order to record *all* coincidence spectrometric data in single runs, even from complex (multielement) biological samples.

5. Bioenergetic Mechanisms Involving Free Radicals

D.C. Borg

Free radicals are chemical forms that possess an unpaired electron, which often can be thought of as an unreacted or "free" chemical bond. In general such molecules are unstable and highly reactive, being especially prone to further oxidations or reductions that yield diamagnetic products containing only paired electrons. As a result, most free radicals have a transitory existence and do not accumulate in high concentrations.

Free radicals are believed to be formed in life processes. The energy derived from foodstuffs is utilized via a series of metabolic oxidations that appear to involve free radical intermediate steps. Active forms of

some drugs may be free radicals, and recent studies have led to the hypothesis that certain hormones are converted to free radical forms in the first stages of some of the biochemical reactions that they initiate.

Furthermore, ionizing radiations are known to produce free radicals in tissue, and much of the radiobiological effect of such radiations is believed to result from further reactions of these radicals. The extent to which these effects are mediated by the free radical mechanisms that are intrinsic to life processes is unknown.

Experimental evidence for the role of free radical forms in the biological actions of hormones and drugs has been elusive, because these substances are active in such small total amounts. Moreover, only a small fraction of these already low concentrations would be expected to exist in the free radical form at any given instant. Therefore, initial efforts to demonstrate the existence of free radical forms of these substances and to study their properties have utilized reaction systems studied *in vitro*. Electron paramagnetic resonance (EPR) spectrometry provides a means of identifying and describing free radicals with great sensitivity and specificity. EPR is a nondestructive technique that is based upon the magnetic properties of unpaired electrons, such as those of free radicals. These paramagnetic properties are not possessed by the usual diamagnetic organic molecules with paired electrons; thus

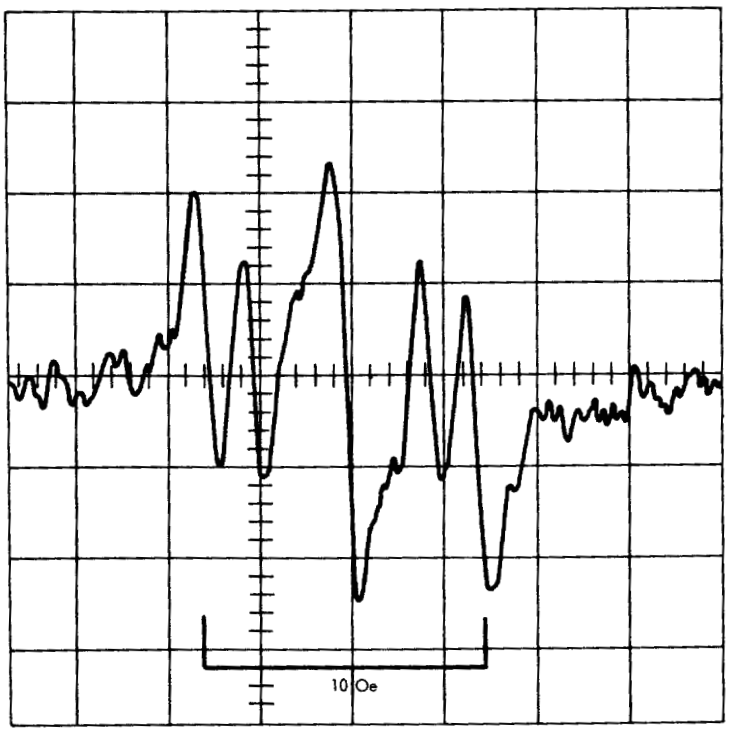


Figure 10. EPR spectrum of the unstable free radical form of the hormone adrenaline. The spectrum was obtained by using the constant-flow technique while reacting the hormone solution with one equivalent of a univalent oxidant in a weakly acid medium.

EPR can be used to study free radicals in the presence of other reactants, including the constituents of living cells.

Recent work here has resulted in improved reaction systems and experimental apparatus for eliciting EPR spectra from labile free radicals. Samples are retained in the liquid state to provide maximum spectral information regarding the electron configuration of free radicals, and constant-flow procedures are used to regenerate short-lived free radical products while EPR is recorded. Dead time between radical production and EPR has been reduced, and previous requirements for relatively large amounts of reacting materials have been lowered. As a result, EPR spectra have been elicited for the first time from free radical forms of biochemical compounds available only in small amounts, such as some hormones.

Data have been obtained describing free radicals from catecholamine hormones (epinephrine, norepinephrine, and synthetic analogues), indole hormones of animals (serotonin, adrenochrome) and plants (indole acetic and butyric acids), thyroid hormones and related analogues, estrogens (phenolic steroids, synthetic estrogenic substances), and from one protein hormone (insulin). Other preliminary investigations showing promise have used the EPR–constant-flow technique to simulate the chains of radiochemical reactions initiated by the action of ionizing radiation upon aqueous systems.

6. Studies on the Applicability of Field Emission Microscopy to Biology

H.A. Johnson

Field emission microscopy, a technique currently useful only in the study of metal surfaces and adsorption phenomena, is being investigated for its possible use with biological materials. It is well known that molecular patterns can be produced by substances sublimed *in vacuo* onto a clean metal surface, but macromolecules of biological interest would have to be deposited on the emitter surface out of solution. The immediate problem, then, is to obtain molecular patterns by using an emitter that is coated by an adsorbed layer of gas or solvent and has a surface structure very likely altered by chemical reaction with the solvent. By comparison of slopes of Fowler-Nordheim curves, it appears that average work functions are not greatly increased by adsorption resulting from contact of platinum with air, water, or acetone. Irregular, multifocal patterns obtained thus far from the evaporation from solution of filamentous molecules of chondroitin sulfate and RNA onto emitter surfaces seem to bear no relationship to molecular structure. Whether these patterns are of any interest or are simply artifactual is yet to be settled.

C. METABOLIC STUDIES

1. Neutron Activation Analysis in Trace Metal Studies Involving Homeostasis

G.C. Cotzias
P.S. Papavasiliou
A. Sakamoto
M.H. Van Woert
K.N. Prasad

For many years clinical interest in manganese was restricted to experimental nutritionists and animal husbandrymen. The recent medical interest is justified on the basis of developments such as the following: (1) Manganese accumulates in mitochondria where it is essential to many steps of oxidative phosphorylation. (2) This metal stimulates the synthesis of cholesterol. (3) Its radioisotope, Mn^{54}, becomes incorporated in hemoglobin heme and can label a single generation of red cells *in vivo*. (4) Treatment of patients or animals with cortisone or some of its analogues induces significant losses of radiomanganese, so that manganese deficiency can no longer be considered as a hazard exclusively to grazing animals and chickens. (5) Chronic manganese poisoning is becoming recognized as a model for the study of human extrapyramidal disease. (6) Melanin granules (such as those lost from the brain in Parkinsonism and in phenylpyruvic oligophrenia) are loci of high accumulation of Mn^{54}.

Clinical investigations were handicapped by the absence of methodology permitting analysis for a trace metal in a small sample of blood, plasma, etc. This handicap appeared difficult to eliminate because manganese is located primarily intracellularly. Neutron activation analysis is a very sensitive technique which permits the avoidance of sample contamination.

Values for manganese in blood, plasma, and cerebrospinal fluid, published from this work, showed the lowest means as well as the smallest degree of scatter among reports available. Since no biological

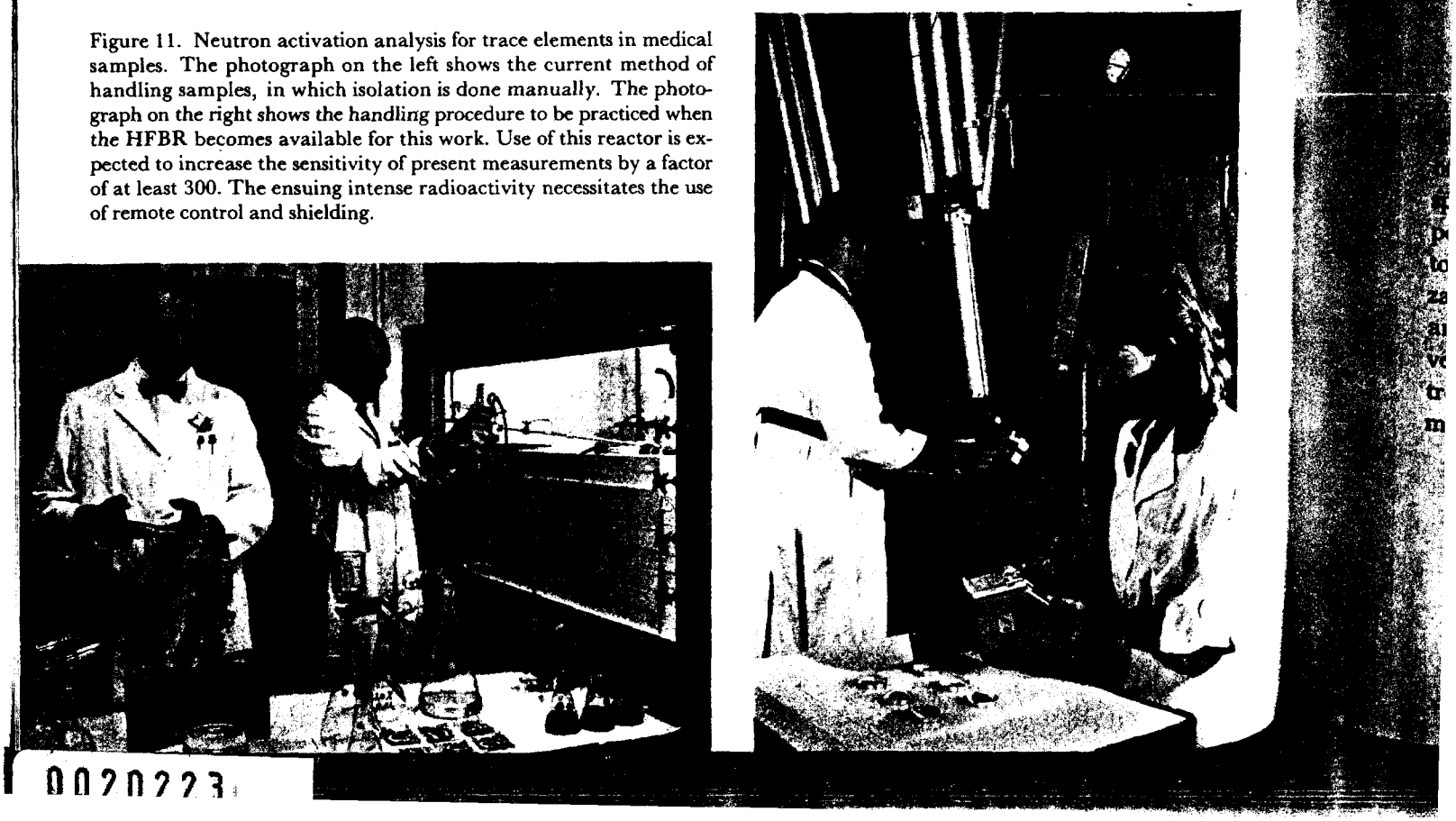


Figure 11. Neutron activation analysis for trace elements in medical samples. The photograph on the left shows the current method of handling samples, in which isolation is done manually. The photograph on the right shows the handling procedure to be practiced when the HFBR becomes available for this work. Use of this reactor is expected to increase the sensitivity of present measurements by a factor of at least 300. The ensuing intense radioactivity necessitates the use of remote control and shielding.

standards are available for intercomparison of results from various laboratories, the thesis has been tested that values from this work might be wrong. Detailed tests now in progress have revealed few and minor sources of possible error, and their elimination lowers the values even more.

Neutron activation analysis has shown the following: The metal's concentration in serum or blood is quite steady from patient to patient and from sample to sample. Its distribution among tissues is altered by surgical stress. Administration of prednisone induces a marked initial rise of plasma manganese, a marked redistribution among various tissues, and significant losses of metal into the feces. These findings support the earlier notion that manganese (and possibly other essential trace metals) is subject to homeostatic control.

Manganese is not excreted by the kidneys, but into the gastrointestinal tract. It was thought therefore that one of the tributaries to the gastrointestinal canal (i.e., biliary excretion) might function as a surrogate kidney in the homeostasis of manganese. Obstruction of the bile flow showed marked accumulation of manganese in the liver and probably also in the muscles of rats. Rats with biliary obstruction seem to lose their ability to regulate the excretion of the dietary manganese absorbed from the gut. Sequential analyses of exteriorized rat bile indicated a striking dependence of the element's biliary excretion on the dietary supply of the element.

The new High Flux Beam Research Reactor (HFBR) at this Laboratory is expected to increase the sensitivity of these measurements by a factor of at least 300. This will permit much higher analytical resolution than hitherto.

2. Studies of Extrapyramidal Diseases

G.C. Cotzias
P.S. Papavasiliou
A. Sakamoto
M.H. Van Woert
K.N. Prasad

The investigations of the physiology of some trace metals seem to have provided tools for the study of selected extrapyramidal diseases of man. Prominent among such diseases is chronic manganese poisoning of miners inhaling manganese ores. This disease often resembles the spontaneously occurring Parkinsonian syndrome. Chronic manganese poisoning is being studied under the joint sponsorship of this Laboratory, the National Institutes of Health, and the World Health Organization, by teams located at the Catholic University of Chile at Santiago and the Medical Research Center at this Laboratory. Techniques developed here will be applied to both affected miners and suitable controls, and the level of manganese in various body pools will be determined by neutron activation analysis utilizing the Brookhaven Medi-

cal Research Reactor. Supplementary utilization of the HFBR as an analytical tool of high resolution may permit analyses of at least 1/300 of the amount of trace metal that can be accurately analyzed at present.

3. *Utilization of Glucose and Intermediate Carbohydrates in Diabetes and Obesity*

W.W. Shreeve
Y. Shigeta
A.J. Tashjian
N. Oji

Further clinical studies of the simultaneous oxidation of glucose-1-C^{14} to $C^{14}O_2$ and of glucose-1-H^3 to H^3OH have been carried out by using the tracer technique. These studies show that obese patients with normal blood sugar but mildly diabetic according to glucose tolerance tests will manifest decreased carbohydrate oxidation under basal conditions as compared with nonobese, nondiabetic subjects. In addition, obese patients with very mild or preclinical diabetes show a definite decrease in the extent of oxidation of DL-lactate-2-C^{14} and pyruvate-2-C^{14} to $C^{14}O_2$, sometimes to one-half that of control subjects. Usually the rate of conversion of DL-lactate-2-H^3 to H^3OH is also depressed in diabetic patients, but results are less consistent than with C^{14}. The decrease in $C^{14}O_2$ from labeled lactate and pyruvate is found to be accompanied by greater than normal amounts of C^{14} converted to glucose and generally slower rates of disappearance of labeled lactate from the blood. These findings emphasize the importance of considering intermediary carbohydrate metabolism in the mechanisms of action of hormones involved in diabetes and obesity. The extent of the abnormalities in formation of $C^{14}O_2$ and H^3OH and the relative ease of measurement of these simple compounds give encouragement to the possibility that sensitive and practical clinical tests for the early or prediabetic state may be developed.

Figure 12. Apparatus for continuous measurement of breath for C^{14} in expired carbon dioxide.

4. *Transfer of Tritium From Various Labeled Carbohydrates to Fatty Acids in Man and Animals* in Vivo

W.W. Shreeve
Y. Shigeta
N. Oji

In earlier studies with diabetic and obese patients it was shown that lactic acid-2-H^3 contributed a larger percentage of its tritium to the formation of fatty acids than did glucose-1-H^3. This has now been confirmed also for the synthesis of total fatty acids of liver and carcass of obese, hyperglycemic mice as well as their lean siblings. Moreover, other sources of hydrogen have been investigated in these mice by the use of malic acid-2-H^3 and isocitric acid-2-H^3. Both of the latter compounds, like glucose-1-H^3, may transfer tritium via the coenzyme nicotinamide-adenine dinucleotide phosphate (NADP), which has been considered most likely to be involved in synthetic reductions. For synthesis of hepatic fatty acids the order of efficiency of labeling is lactic acid > malic acid > glucose > isocitric acid. Since lactic acid dehydrogenase typically utilizes the coenzyme NAD (rather than NADP), the preference for NADP as a coenzyme for fatty acid synthesis does not appear to obtain in the liver *in vivo*. For carcass fatty acids, however, glucose-1-H^3 is superior to the other labeled compounds for synthesis of fatty acids. Studies in which these compounds are labeled with both H^3 and C^{14} have been initiated to provide another index of specificity of the various sources of metabolic hydrogen.

Studies with all the H^3- or C^{14}-labeled carbohydrates tend to confirm the earlier observation with glucose-1-H^3-1-C^{14} that the obese mice transfer 5 to 10 times as much of the radioisotopes to hepatic fatty acids as do their lean littermates. With carcass fatty acids the differences are generally less, i.e., the obese mice transfer 2 to 4 times as much as their lean littermates. These studies strongly suggest that the grossly fatty livers of the obese mice, as well as their general obesity, are related to a marked hyperlipogenic abnormality. The latter may be induced by excessive pancreatic secretion of insulin, which, however, does *not* exert its effect by providing hydrogen for fatty acid synthesis specifically by accelerating glucose metabolism via the pentose pathway.

5. *Studies With C^{14} of Pharmacological Effects on Carbohydrate Metabolism in Cerebral Cortex* in Vitro

J. J. O'Neill
W.W. Shreeve

The effect of artificial electron acceptors and agents which "dissociate" or "uncouple" oxidation from phosphorylation on the various pathways of glucose metabolism is being studied in slices of brain cortex of guinea pig with glucose-1-C^{14}, glucose-6-C^{14}, and glucose-*U*-C^{14}. The cortical slices are set up for artificial electrical stimulation to simulate activity *in vivo*. Since the operation of the pentose pathway of glucose metabolism provides more direct and faster formation of carbon dioxide than does the Embden-Meyerhof glycolytic pathway, comparisons of rate and extent of formation of $C^{14}O_2$ from the various spe-

cifically labeled glucose compounds permit certain deductions about the relative activities of these pathways. Studies with such artificial electron acceptors as phenazine methosulfate, methylene blue, and paraphenylene diamine in the incubation medium with the labeled glucose and electrically stimulated tissue demonstrate utilization of the first carbon atom at rates up to ten times the apparent rate of utilization of the sixth carbon atom. This reveals a possible latent capacity for the operation of the pentose pathway if sufficient electron acceptors are present. "Uncouplers," such as dinitrophenol, and the imposed electrical stimulation itself increase the rate of metabolism of glucose, but show no preferential formation of carbon dioxide from the first carbon atom. Further studies are designed to isolate various glycolytic intermediates in the slice in order to locate enzymatic steps in which there is stimulation or inhibition by various drugs and other agents.

6. *Effects of Ethanol on Intermediary Metabolism*

S.M. Joubert
W.W. Shreeve
A.J. Tashjian

Recognition of alcoholic hypoglycemia as a clinical entity has stimulated interest in the mechanisms involved in its genesis. The oxidation of large amounts of ethanol in the cell is known to decrease the ratio of oxidized to reduced coenzymes. This could influence the synthesis of glucose from 3-carbon precursors, since the metabolic pathways include both oxidative and reductive steps using these coenzymes. By analyzing the distribution of C^{14} in glutamic acid from liver proteins after administration of alanine-2-C^{14}, it is possible to study changes in relative reaction rates of these metabolic pathways and to determine the effect of the disturbed coenzyme redox potentials. Investigation of the C^{14} distribution under various conditions of fasting, glucose load, and/or ethanol load in the rat have indicated that ethanol load probably does not suppress the pathway through dicarboxylic acids for gluconeogenesis from alanine.

Ethanol oxidation could decrease gluconeogenesis if it curtailed the supply of 3- or 4-carbon substrates by using them as a "sink" for the reducing equivalents, e.g., converting pyruvate to lactate. A further study of the distribution of H^3 in glucose after administration of ethanol-1-H^3 has been undertaken to investigate this point. A characteristic distribution would indicate whether the tritium is passed on through lactate, malate, phosphoglycerate, or some other substrate.

Another new means of investigating the nature and degree of disturbance of the hydrogen-transferring coenzymes is the study of synthesis of the coenzymes by the use of nicotinamide-7-C^{14}, a metabolic precursor of the pyridine moiety of these nucleotides. By this technique

the rate of turnover between the oxidized and reduced forms is being studied in rats.

7. *Studies of the Neurohormonal Role of Glucose in Appetite Regulation*

P.M. Edelman
I.L. Schwartz
E.P. Cronkite
G. Brecher
W.W. Shreeve
E. Lamdin

The hypothalamic regulation of feeding behavior involves a continually active lateral feeding center which induces a sustained urge to eat, and a normally quiescent ventromedial satiety center which, when activated, suppresses this urge. It has been suggested that the satiety signals originate as a result of uptake or metabolism of glucose by the cells of the ventromedial center. This glucostatic theory of appetite regulation derived support from our previous observation that gold could be accumulated in the region of the ventromedial satiety center when coupled to glucose, as in gold thioglucose, but *not* when coupled to other substrates, as in gold thiomalate.

Specific uptake of gold thioglucose in the ventromedial hypothalamus could be due either to a selective permeability to glucose and gold thioglucose of the blood-brain barrier or to the presence of specific glucoreceptor sites with high affinity for both glucose and gold thioglucose. If the selectivity is due to fixation of gold thioglucose at glucose binding sites, it should be possible to demonstrate a competitive suppression of gold thioglucose uptake by raising the ratio of glucose to gold thioglucose molecules presented to such glucoreceptors. To test this point, steady-state levels of hypoglycemia and hyperglycemia were established in several groups of mice so that animals having widely varying levels of blood glucose concentration could be injected with a standard dose of gold thioglucose. It was found that hyperglycemia did not diminish the focal deposition of gold in the hypothalamus but, in fact, increased it. Thus, glucose and gold thioglucose do not appear to compete for attachment in the ventromedial nucleus; i.e., they do not appear to share a common receptor. Therefore, the gold accumulation and lesions cannot be due to binding of gold thioglucose by specific glucoreceptors, and the glucostatic triggering of the satiety response probably depends on selective permeability to glucose of the blood-brain barrier. The principal analytic method used in this study depended on neutron radioactivation of Au^{197} which made possible determination of as little as 2×10^{-11} moles of gold in tissue.

A new appetite-suppressing drug, α,α-methylchlorphentermine, which is active specifically on the hypothalamic appetite centers, is being studied for its over-all metabolic fate by administration to obese patients of a C^{14}-labeled sample of this drug followed by analysis of breath, urine, blood, and feces for the drug or its metabolic products

tagged with C^{14}. No radioactivity has been found in expired $C^{14}O_2$. Urinalyses have shown that 15% of the drug (or its products) is excreted by this route within 24 hr after oral administration in a rapid-release form, 60% after 7 days, and 75% after 14 days.

8. *Studies of the Relation of Chemical Attachment to Physiologic Action of Neurohypophyseal Peptides on Membrane Phenomena*

I.L. Schwartz
E. Lamdin
L. Livingston
L. Rahm
M.S. Schoessler
D.C. Goldberg
P. Eggena
C. Hirsch

In order to obtain H^3-labeled neurohypophyseal peptides of increased specific activity, particularly for studies of subcellular sites of localization employing high resolution radioautography, attempts to modify the Wilzbach procedure have been continued. In addition, a program of peptide synthesis has been initiated as an additional means of obtaining labeled peptides and for selected studies of the relation of chemical structure to the affinity and intrinsic activity of antidiuretic hormone.

The interaction of tritium-labeled oxytocin with the rat kidney was investigated in order to get information about the chemical specificity of the antidiuretic action of mammalian neurohypophyseal hormones. Tritium-labeled oxytocin was prepared and injected into the renal arteries of ethanol-anesthetized rats in dosages ranging from 10^{-9} to 10^{-8} g. No antidiuretic action was noted in this dosage range, and no evidence of sulfur-sulfur linkage to renal tissue was observed after homogenization and treatment of washed labeled kidney sediments by methods that cleave SS bonds but do not disrupt peptide bonds (0.1 *M* cysteine, *p*H 8; 0.05 *M* sulfite in 6 *M* urea, *p*H 7). Control studies using H^3-lysine vasopressin showed SS linkage of hormone to tissue at all dosage levels, with direct correlation between binding and antidiuretic activity in the lower dosage range. These findings support the hypothesis that the formation of SS bridges between the neurohypophyseal peptides and kidney tissue represents a functional hormone-receptor interaction.

In a continuing collaboration with Dr. H. Rasmussen of the University of Wisconsin, an analysis of the effect on membrane permeability (in the toad bladder) of 35 analogues of arginine vasotocin was concluded. The data in conjunction with studies of the *p*H dependence of the response of the bladder to hormone also supported the suggestion that an SH-SS interchange reaction involving receptor SH and hormonal SS initiated the chain of events leading to the increase in membrane permeability.

In another series of studies, intrinsic and extrinsic factors that tend to diminish activity of the target organ (again the toad bladder) to hormone were identified and shown to be related to the history of pre-

vious hormonal challenge, particularly with respect to dosage, duration, and frequency. Studies have also been conducted in which changes in composition of the bathing medium were found to evoke reproducible changes in reactivity to hormone. These changes are attributed tentatively to alterations in the conformation of the receptor molecules.

9. Studies of the Chemical Attachment of Insulin to Muscle and Adipose Tissue

P.M. Edelman
S.L. Rosenthal
J. Edelman
I.L. Schwartz

During the course of an investigation of the attachment and intracellular localization of I^{131}-labeled iodoinsulin, a method was developed for the isolation of the muscle cell sarcolemma (Figure 13). When whole muscle was exposed to I^{131}-insulin, the isolated muscle cell membranes were found to be extensively labeled, the radioactivity being bound both through electrovalent and covalent (SS) linkages. The resolution of I^{131}-insulin preparations into A and B chains and the determination of the intramolecular localization of the radioactivity showed that the initial insulin-tissue reaction involved the formation of an SS bond between the membrane protein and the half-cystine moiety in position 6 and/or position 11 on the A chain of the hormone. (Another possibility not excluded is that the A and B chains attach separately to discrete receptor sites.) In the course of this work muscle tissue was separated by differential centrifugation into nuclear, mitochondrial, microsomal, and soluble components in order to determine the distribution of I^{131}-insulin within the muscle cell. The preparation of isolated skeletal muscle cell nuclei (Figure 14) will be used for study of the reactions between insulin (and other hormones) and nuclear components and for studies of hormonal and other factors involved in the control of nuclear protein synthesis.

Figure 13. Isolated sarcolemma.

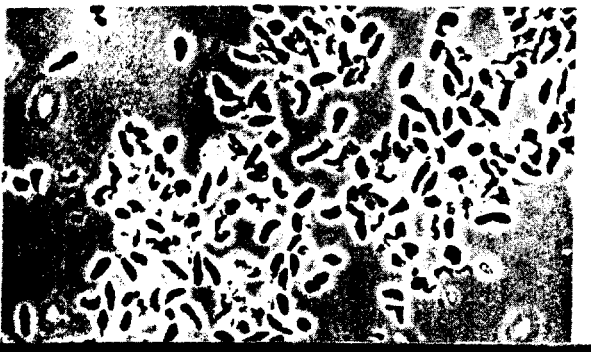


Figure 14. Isolated muscle cell nuclei.

C. METABOLIC STUDIES

10. Interrelationship of Sodium and Genetics With Hypertension

L.K. Dahl
E. Schackow
L. Lax
C. Young

These studies are oriented toward the significance of the role of sodium in the pathogenesis of human hypertension. Some of the evidence on which this thesis rests is as follows: experimental hypertension can be induced in rats by chronic excess salt ingestion; as the average daily salt intake is increased, the frequency and severity of hypertension in the rats increases; and, in conformity with this observation in animals, it has been found in five different communities in the world that the prevalence of hypertension in human beings increased linearly as the average daily salt consumption in the respective communities increased. However, it has been observed repeatedly, both in man and in rats, that some individuals failed to develop hypertension despite life-long high salt intakes. In contrast, at least in the experiments with rats, some animals died of fulminating hypertension a few months after commencing high salt diets. These marked variations in response to similar amounts of the same agent suggested that differences in genetic constitution were involved. During the last four years this possibility has been explored with the technique of selective inbreeding. Two strains of rats have been evolved that differ markedly in their response to salt: one strain, the so-called Sensitive Strain, rapidly develops severe and fatal hypertension from a salt intake to which the other, the so-called Resistant Strain, responds hardly at all. Similar variations in genetic sensitivity to salt in man would explain, at least in part, the observed fact that whereas some human beings are remarkably responsive to increments and decrements of salt, others are notably unresponsive.

Despite the fact that salt consumption precipitates hypertension in rats of the Sensitive Strain, the subsequent restriction of salt more often than not fails to cause a fall in blood pressure. Furthermore, recent studies have failed to reveal increased sodium in the tissues of these salt-fed hypertensive rats as evidenced (1) by carcass analysis, (2) by total exchangeable sodium (measured with either Na^{22} or Na^{24}), or (3) by differences in the biological half-life of Na^{22} in animals with and without hypertension. Thus, while a high salt intake may initiate the hypertensive process, continuation of this salt intake is not required for maintenance of hypertension, and there is no evidence of gross accumulation of sodium in the hypertensive animal. Furthermore, a number of hypertensive human beings who did not have a prolonged biological half-life for Na^{22} have now been observed. This study was an extension of earlier studies in man in which a statistically significant increase in the biological half-life of Na^{22} among hypertensive subjects was observed. The exceptions found recently suggest that such an increase is not a necessary correlate of the disease, and this finding is in keeping with the observation in rats.

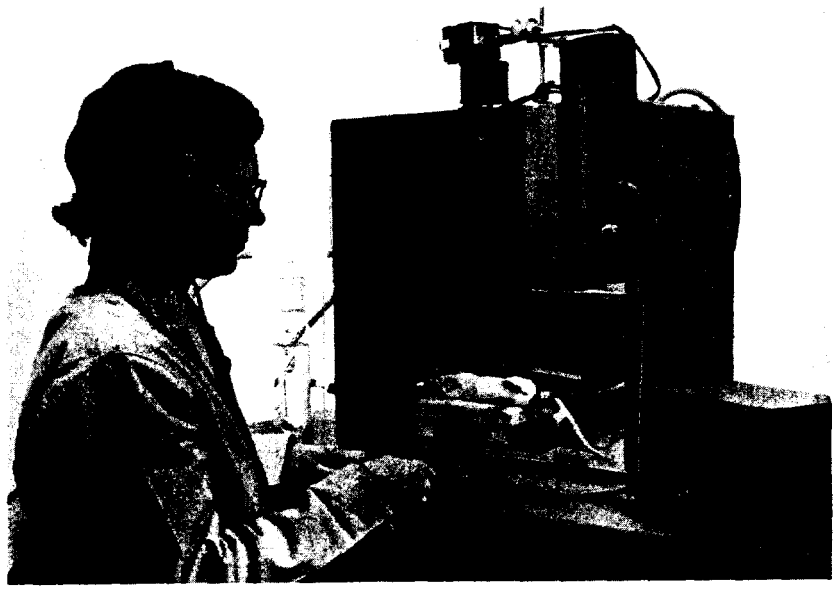


Figure 15. Measurement of blood pressure in rats made hypertensive by salt feeding.

In patients with hypertension, vascular hyperreactivity is usually present, but it has always been uncertain whether this preceded or followed the disease. If it preceded the onset of frank hypertension, this could play an important role in the development of the high blood pressure. Normotensive rats of the Sensitive Strain have shown an increased blood pressure response to vasoconstrictor agents before salt feeding as compared with animals of the Resistant Strain. It seems probable that innate vascular hyperreactivity is part of the genetic component that characterizes those rats destined to become hypertensive and that this may have a bearing on the process of essential hypertension in man.

11. DL-*Tryptophan Metabolism in Human Beings*

L.V. Hankes
R.R. Brown
E.P. Cronkite

The studies of DL-tryptophan metabolism in patients were expanded to include anemia patients. As in previous studies, the patients were placed on a controlled diet (minimum permissible tryptophan intake of $\approx$1.2 g/day) 7 days prior to the administration of an oral dose of 2 g L-tryptophan. The patients ranged in age from 21 to 52 years.

Urine analysis for kynurenic acid, xanthurenic acid, indican, anthranilic glucuronide, *o*-aminohippuric acid, acetylkynurenine, kynurenine, and hydroxykynurenine showed no abnormal excretion of these urinary products in the patients studied.

C. METABOLIC STUDIES

As a therapeutic measure for one patient (21 years old) with microcytic hypochromic anemia whose urinalysis showed very high urinary quinolinic acid, N^1-methylnicotinamide, and N^1-methyl-2-pyridone-5-carboxamide hydrochloride levels, vitamin B_6 was administered (100 mg, three times a day) for a period of 2 wk and the 2 g L-tryptophan overload test repeated. The high levels of urinary quinolinic acid, N^1-methylnicotinamide, and N^1-methyl-2-pyridone-5-carboxamide hydrochloride were lowered significantly after vitamin B_6, and the increase (after tryptophan overload) in the levels of these urinary components was much less than that observed prior to the vitamin B_6 treatment. The urinary excretion data suggested a relationship between quinolinic acid production and vitamin B_6. The straight chain compound (1-amino-4-formyl-buta-1,3-diene-dicarboxylic acid) formed when the ring of 3-hydroxyanthranilic acid is split, apparently requires vitamin B_6 for its metabolism down to CO_2, and when the tissue B_6 level is low or its utilization is blocked, the 1-amino-4-formyl-buta-1,3-diene-dicarboxylic acid cyclizes to form quinolinic acid, which appears as an increased level of quinolinic acid (and its products) in the urine.

To determine whether a definite relationship exists between vitamin B_6 and quinolinic acid levels, a study of the effect of vitamin B_6 deficiency in urinary quinolinic acid levels in the human was initiated on a collaboratve basis with Dr. R.R. Brown at the University of Wisconsin.

12. DL-Kynurenine-Keto-C^{14}, Synthesis and Resolution

L.V. Hankes

An abbreviated synthesis of DL-kynurenine-keto-C^{14} was developed which improved the yield over an older method by a factor of 4. The DL-kynurenine-keto-C^{14} was isolated as the sulfate and it had an activity of ≈ 1 μC/mg.

Small quantities of the DL-kynurenine-keto-C^{14} were placed on a large paper pulp column (10×130 cm), and the column was developed with a solvent system of methanol:benzene:butanol:water (2:1:1:1) with 1% acetic acid added. The labeled DL-kynurenine separated into 2 ultraviolet fluorescent bands of L-kynurenine-keto-C^{14} and D-kynurenine-keto-C^{14}, which were collected in 0.50 to 100-ml volumes of eluate. Paper chromatography of the eluates showed an efficient separation of the two isomers.

Figure 16. Oversized paper pulp column used to separate small (300-mg) quantities of DL-kynurenine-keto-C^{14} into the D and L isomers for use in cancer research.

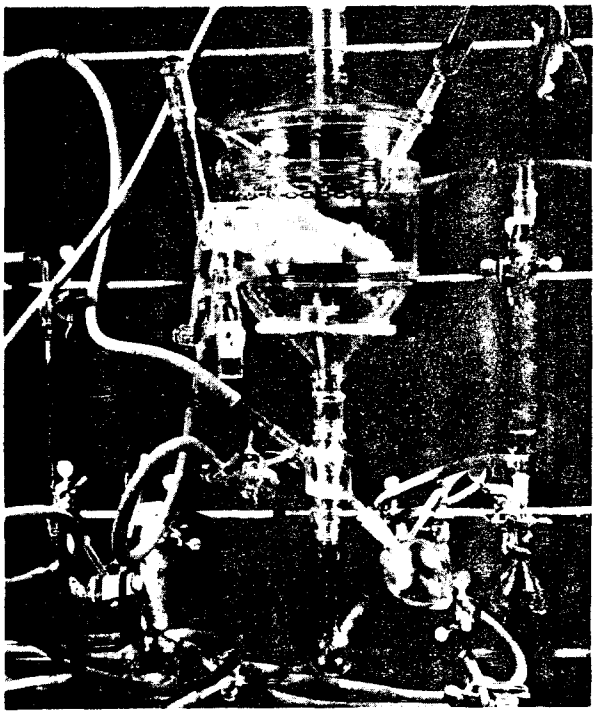


Figure 17. Animal metabolism apparatus used to collect $C^{14}O_2$ and C^{14} urinary components from animals injected with C^{14}-labeled compounds.

13. 3-Hydroxyanthranilic Acid-Carboxyl-C^{14} Metabolism in the Human

L.V. Hankes
R.R. Brown
S.W. Lippincott

The tryptophan metabolite 3-hydroxyanthranilic acid has been established as an intermediate in the conversion of tryptophan to niacin. The recent observations of elevated levels of urinary hydroxyanthranilic acid in patients with bladder cancer and the confirmed carcinogenicity of hydroxyanthranilic acid for the mouse bladder have focused attention on this and other tryptophan metabolites. A disorder of tryptophan metabolism was shown in which abnormal levels of aromatic amines derived from tryptophan were found in the urines of patients with spontaneous bladder cancer; such levels were not found in the urines of patients with bladder cancer caused by exposure to industrial aromatic amines. Abnormal urinary levels of the aromatic amines have been detected in a number of other diseases. In view of these observations it was deemed essential to determine how the human would metabolize labeled hydroxyanthranilic acid as compared to other animals.

A female patient with achondroplasia who had previously been found to have normal tryptophan metabolism, as determined by quantitative urinary analysis for a number of tryptophan metabolites before loading with 2 g L-tryptophan, was given orally 14.088 mg of carboxyl-labeled 3-hydroxyanthranilic acid containing 51.055 μC C^{14}.

The expiration of $C^{14}O_2$ by the patient was similar to that observed in rats, except that a smaller percentage of the dose was expired as $C^{14}O_2$. Studies with DL-tryptophan-2-C^{14} in humans have shown that the $C^{14}O_2$ levels in the expired air will change from 5% to $\approx$25% if the dose is increased from 40 mg to 2 g.

The percentage of urinary C^{14} quinolinic acid was found to be much higher in man as compared to the rat, which suggests some quantitative difference in the manner in which hydroxyanthranilic acid is metabolized in the two species. Of the activity in the patient's urine, it was possible to account for 16 μC of the 18.54 μC excreted over a 72-hr period as quinolinic acid, N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxylamide, and nicotinic acid. The order of magnitude of percent of activity present in each isolated urinary component suggested that renal clearance of compounds as well as dosage administered plays a role in the over-all results obtained with labeled compounds. The normal pathway of hydroxyanthranilic acid metabolism was assumed to be via quinolinic acid, nicotinic acid, N^1-methylnicotinamide, and then pyridone; therefore, it was noteworthy that the order of components excreted was quinolinic acid, pyridone, N^1-methylnicotinamide, and nicotinic acid.

The total radioactive content of the serum seemed to consist of at least two components, a major portion which does not precipitate with the serum proteins, and a minor portion which is precipitated. This bound activity decreased with time at a much slower rate than did the soluble activity. The chemical nature of the protein-bound radioactive component was not determined; however, it may result from incorporation of breakdown products of hydroxyanthranilic acid or intact hydroxyanthranilic acid. It is possible that this kind of tissue-binding of hydroxyanthranilic acid or its metabolites may play a role in carcinogenesis.

14. Protein Metabolism in Malignancy

J.E. Jesseph
J.L. Bateman

It has been found that with progression of certain malignancies, particularly cancer of the breast, there is a more rapid catabolism of I^{131}-labeled human globulins. The rate of catabolism is estimated by serial whole-body gamma spectrometry of patients after intravenous administration of a small amount of the labeled protein. The biological half-time measured by this method has been found to approximate 13 days in normal control subjects and in patients with stable, chronic diseases without evident effect on protein catabolism. In patients with progressing, uncontrolled malignancies, on the other hand, the half-time is reduced to an average of $\approx$6.5 days; thus the exogenous tracer-labeled globulin is destroyed twice as readily as in the "normal" individual.

In order better to relate this process to the malignant state, turnover studies during the past year have been performed prior to, and at

intervals during, courses of combined chemo- and radiotherapy of patients with metastatic cancer. In particular, it was of interest to determine whether the catabolism of labeled homologous protein, accelerated with advancing disease, would revert toward normal in patients brought into clinical control or remission. Treatment was individualized for each patient and regulated by the degree of response to provide the greatest likelihood of useful palliation, leading to rehabilitation.

Results to date with this latter group of patients have shown a tendency for half-time of globulin to return toward the normal value of $\approx$13.0 days. An average of $\approx$10.0 days has been obtained in patients who have responded to treatment with healing of bone destructive lesions and regression or disappearance of malignant fluid accumulations and tumor masses. Return of appetite, weight gain, and major reduction in symptoms have usually accompanied the above changes.

The significance of the apparent relationship of homologous protein catabolism to degree of malignant involvement is not clear. It is possible that the turnover rate of this material reflects the status of the body's defense mechanism against homologous proteins, or it may reflect a nonspecific alteration of protein metabolism in general.

15. Metabolism of Nucleic Acids

S.L. Commerford

When the thymidine analogue iododeoxyuridine (IDU) is administered to mice, some is incorporated into those DNA molecules being synthesized at that time and the rest is rapidly broken down and excreted. By labeling IDU with a gamma-emitting isotope of iodine, it becomes possible to measure the amount of IDU incorporated into DNA at any time by external measurement of gamma-ray activity. Since DNA does not leave the cell except upon death of the cell, the use of labeled IDU provides a simple, nondestructive method for measurement of cell proliferation and death.

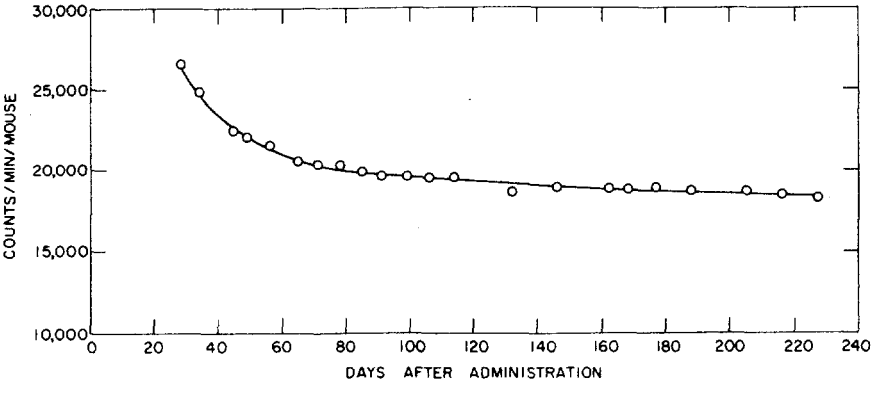


Figure 18. Whole-body retention of I^{125}-labeled IDU given 5 days before birth. Each point represents the average value for 10 mice. Data have been corrected for radioactive decay.

C. METABOLIC STUDIES

In order to label cells with little or no turnover rate in the adult, it is necessary to administer the labeled IDU at a time when these cells are rapidly proliferating, i.e., just before or soon after the mouse is born. At present this is being accomplished by injecting the label into pregnant mice 1 to 5 days before parturition. Nearly all the tissues in the entire litter are found to be labeled. However, as time passes, the cells with a short life-span die, their label is excreted, and they are replaced by unlabeled cells, so that by the time these mice are 2 mo old, >80% of their label is present in cells with a life-span >1 yr. The determination of the distribution and longevity of these cells in various tissues and the effect of ionizing radiation and mutagenic chemicals on the viability of these cells is in progress.

16. Cell Proliferation in High Oxygen Atmospheres

R.M. Drew
R.B. Painter
L.E. Feinendegen

The metabolic changes and sequelae associated with prolonged breathing of gaseous mixtures of high oxygen content have long been recognized but have demanded more intensive investigation during the past decade because of problems encountered in space travel and in new approaches in radiotherapy which make use of high oxygen tension. Although the requirements for man's atmospheric environment will vary for submarine, terrestrial, and transatmospheric adventures, oxygen will be common to all. There is, therefore, the need for a broader understanding of the toxic effects of oxygen on various body systems, particularly with reference to the time-concentration relationships.

Advantage has been taken of some of the newer radioisotopic materials to study the replication of tissue cells in culture in high O_2 environments. HeLa S3 cells can be readily cultured in nutrient medium

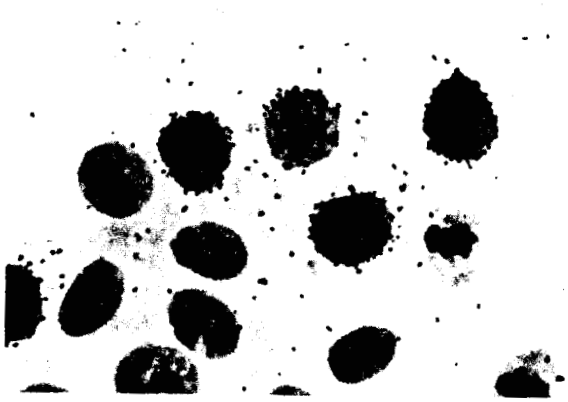


Figure 19. H^3-thymidine incorporation by cells cultured in 95% air + 5% CO_2 for 24 hr.

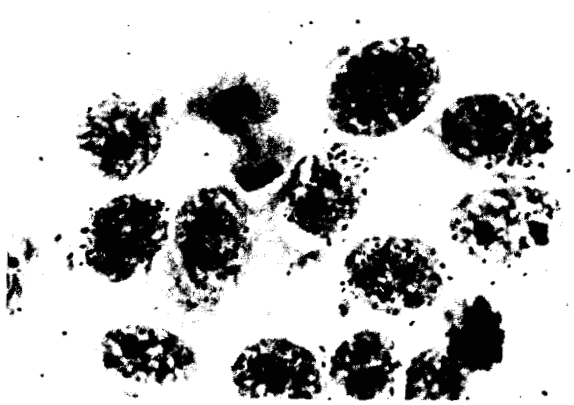


Figure 20. H^3-thymidine incorporation by cells cultured in 95% O_2 + 5% CO_2 for 24 hr.

at 37°C in an atmosphere of 95% air +5% CO_2 with an average generation time of 22 hr. However, if the cultures are incubated in either 50% O_2+5% CO_2+45% N_2 or 95% O_2+5% CO_2, there is evidence of a cytotoxic effect within 48 hr. Granulation and stretching of the cells first occurs, followed by a "rounding-up" and detachment from the culture vessel wall. The detached cells cannot be subcultured even under the most favorable condition.

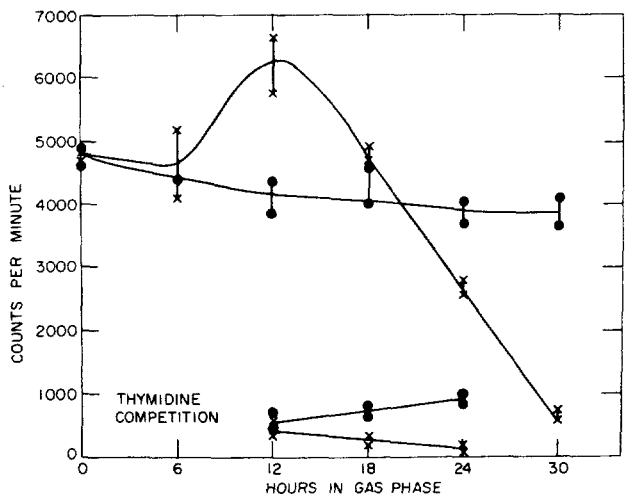


Figure 21. I^{131} activity of DNA extracts. *X*, cells cultured in 95% O_2 + 5% CO_2; •, control cultures in 95% air + 5% CO_2.

Tracer studies with H^3-thymidine have revealed that the DNA synthetic period is somewhat prolonged and that there is a lag in the appearance of labeled mitotic figures in cell populations cultured in high O_2 atmospheres. A study of the incorporation of radioactive DNA precursors by the cells at various times during growth in high oxygen environments indicates that the oxygen treatment exhibits few effects on DNA metabolism during the first 12 hr. However, at this time the cells exhibit significantly enhanced incorporation of either H^3-thymidine or I^{131}-labeled deoxyuridine. The simultaneous addition of nonradioactive thymidine suppresses the burst of incorporation. Because of the higher utilization of H^3-thymidine or I^{131}-labeled deoxyuridine beginning at $\approx$12 hr and the competitive action of thymidine, the present concept is that a depression of specific DNA precursors in the pool occurs, or that the cells are unable to synthesize nucleotides and nucleosides at the normal rate under oxygen stress.

The rest phase after mitosis and preceding DNA synthesis (G_1 period) is known to be 12 to 14 hr for HeLa S3 cells cultured in 95% air +5% CO_2. Since the effects of high O_2 are not evident for $\approx$12 hr, it is interesting to speculate on the possibility that only those cells that enter the G_1 period after the onset of high O_2 treatment are affected.

17. *Prevention of Iron Accumulation in Aregenerative Anemia*

L.M. Schiffer

As a part of studies on iron and vitamin B_{12} metabolism, the effect of anemia *per se* in iron absorption was investigated. A considerable number of anemic conditions are amenable to therapy only by blood transfusion. One of the many problems in treating patients with aregenerative anemia is the gradual, but incessant, increase of iron stores, brought about by the limited ability of the body to excrete iron. The iron that is administered as an integral part of the hemoglobin of the transfused blood is eventually stored in the liver, spleen, pancreas, and other organs and may then play a part in further deterioration of the patient's condition.

In recent experiments with animals made anemic by exposure to Sr^{89}, it was shown that anemia *per se* influences iron absorption. To determine whether this holds for human beings, seven patients with aregenerative anemia were given iron absorption tests when anemic, and when hemoglobin was maintained at normal levels with transfusions. The iron absorption procedures, using Fe^{59} and the whole-body gamma spectrometer, showed that in six of the patients iron absorption was significantly greater during the anemic period.

These studies point to a basic mechanism involving perhaps a humoral factor in iron absorption; or the results may reflect the response of the bowel to hypoxia.

Desferrioxamine *B*, a siderochrome chelating agent, will remove from the body 25 to 50 times the amount of iron normally excreted. The effect of this agent on excretion of Fe^{59}-labeled stores is under study. At present no curative agent for these disease states is known, but it is hoped that prevention of iron accumulation will prolong useful life beyond its present limits.

D. CELL PROLIFERATION KINETICS STUDIES

1. *Incisor Growth and Cellular Behavior Followed Autoradiographically With Tritiated Thymidine*

W.S. Hwang
E.A. Tonna
E.P. Cronkite

Tritiated thymidine was used in autoradiographic studies of the proliferative potential of the cellular complement of mouse incisors and the migration rate of ameloblasts in 5-wk-old female mice. Preameloblastic and preodontoblastic populations exhibited the highest labeling indices, 0.292 and 0.290, respectively. One hour after H^3-thymidine administration, no labeled ameloblasts and odontoblasts were observed. Labeling indices of pulp cells and fibroblasts in the periodontal membrane were small. The migration rate of ameloblastic cells, measured

at intervals from 1 hr to 24 days, was found to be linear with time after 16 hr. A period of 24 hr appeared to be required for ameloblasts to complete proliferation and maturation and to reach the functional zone. The average migration rate of ameloblasts was 365 μ/day during the observation period of 24 days. Appearance of globular structures in the stratum intermedium and ameloblastic layer indicated degeneration of labeled ameloblasts nine days after maturation. The average daily growth of the incisor was measured grossly (at 338 μ/day) and correlated with the migration rate of ameloblasts assessed microscopically (at 365 μ/day). Similar studies are being carried out on older mice.

2. Skeletal Growth and Aging Studied Autoradiographically With Tritiated Amino Acids

E.A. Tonna
E.P. Cronkite

H^3-histidine and H^3-glycine were used in a number of studies of the cellular phase of the femora of young and old mice. The degree of participation and turnover of label by different cell types during matrix formation was assessed autoradiographically. In addition, growth dynamics of the femur was studied by observing the movement of the matrical label which accumulated in the form of a band.

Following administration of a H^3-amino acid the label was first taken up by cells and later deposited within new matrix. This observation corresponds to the intracellular formation of matrix precursors and their liberation during matrix production. In 5-wk-old mice the uptake was highest in metaphyseal endosteal osteoblasts and cartilage cells of the epiphyseal plate, then in periosteal osteogenic cells, and finally in cartilage cells of the articular surfaces. In 1-yr-old mice whose epiphyseal plate was closed, only metaphyseal endosteal osteoblasts re-

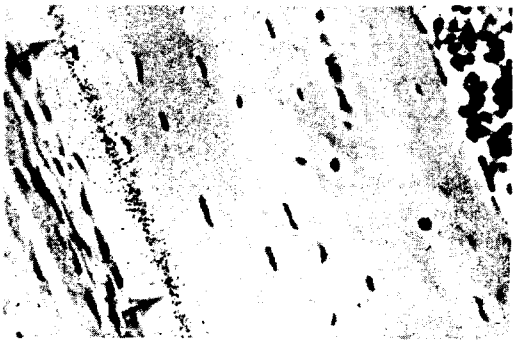


Figure 22. An autoradiogram of mouse femoral cortical bone 4 days after H^3-glycine administration, showing the movement (arrows) of the band of silver grains as more unlabeled periosteal bone matrix is deposited upon the labeled bone. Note that in this region the endosteum of the cortical bone is not participating in this activity (compare with Figure 23).

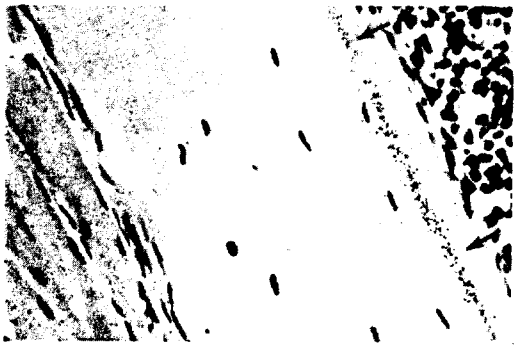


Figure 23. An autoradiogram of mouse femoral cortical bone 4 days after H^3-glycine administration, showing the movement (arrows) of the band of silver grains, this time from the endosteum, as more unlabeled bone matrix is deposited. Note that in this region the periosteum is inactive.

vealed activity reminiscent of young animals, while the remaining cell types revealed very diminished uptake. A semilogarithmic plot of the grain count data on young mice revealed the existence of a 2-component exponential system. The fast component (in hours) is representative of the turnover of matrical protein precursors, and the slow component (in days) represents turnover of protoplasmic proteins. In old mice the 2-component system is absent except in metaphyseal osteoblasts. The single component is similar in magnitude to the slow component of young mice and largely represents protoplasmic protein turnover.

It was concluded from these data that the turnover rate of structural, protoplasmic proteins is less sensitive to changes with time and tissue environment than is the turnover rate of specific compounds required for special cell function.

3. Lymphoid Cells in the Dog Bone Marrow and Blood

G. Keiser
B. J. Bryant
H. Cottier
V.P. Bond

The lymphoid cells of dog bone marrow and blood are of two types: (1) pachychromatic cells of lymphatic origin, and (2) leptochromatic cells, similar to the transitional cells described by Yoffey and Harris, of controversial origin (lymphatic vs. bone marrow). In the nonirradiated dog, most of these cells are small. During the early phase of recovery from sublethal whole-body x irradiation, the marrow cellularity is poor and is marked by the presence of large numbers of lymphoid cells. Most of these cells are leptochromatic, and, in comparison to normal dogs, the relative proportion of medium and large leptochromatic cells is increased. The kinetics, origin, and fate of lymphoid cells in normal and irradiated dog bone marrow and blood were studied by the following method: Circulation arrest of one or both hind legs was achieved by clamping the femoral artery and vein. Immediately thereafter, H^3-thymidine (1 mC/kg body weight) was injected into a front leg or sublingual vein. Serial smears for autoradiography were then made of venous blood and of bone marrow aspirated from different parts of the clamped leg (experimental marrow) and other parts of the skeleton (control marrow). The effectiveness of clamping was attested by the absence of labeled cells in the experimental marrow and of radioactivity in the plasma of the clamped leg prior to release of the clamps at 40 min after isotope injection.

The following results were obtained for the nonirradiated dog: Heavily labeled leptochromatic lymphoid cells of medium and large size were found in the blood and control marrow within 15 min after H^3-thymidine injection. These cells began to accumulate in the experi-

mental marrow within 5 min after release of the clamps. Heavily labeled small lymphoid cells began to appear in the experimental marrow somewhat later, at 2 to 3 hr post H^3-thymidine, and reached a maximum on days 2 to 6. Their labeling indices (maximal values, 15 to 20%), however, were significantly lower than those of the control marrow (maximal values, 25 to 30%). After 7 hr, erythroblasts of all stages of maturation had a very low labeling index and mean grain count, whereas the latter was significantly higher in the control marrow up to day 3. On the other hand, the mean grain count of the erythroblasts of the experimental marrow was significantly lower than that of lymphoid cells during the first two days.

The data suggest that the dog bone marrow may contain two populations of small lymphoid cells, one originating from initially labeled precursors within the marrow (larger transitional cells?), the other immigrating from the blood. No evidence has been obtained that the lymphoid cells which have migrated can transform into hemopoietic cells within two days.

In comparison, the following results were obtained for the irradiated dog: Clamping and H^3-thymidine procedures were carried out during the early recovery phase (days 6 to 10) following whole-body x irradiation with 250 r. At similar time intervals in both normal and irradiated dogs, labeled lymphoid cells were observed in the blood and in both the experimental and control marrows. In the irradiated dog, however, the labeling index of these cells exceeded 40% on day 1 following H^3-thymidine injection in the blood and in both marrows. During the first 24 hr, labeled erythroblasts were found only in the control marrow. On subsequent days, however, the experimental marrow showed labeling in some unidentifiable blast-like cells. Although the high grain count of these cells largely excludes their labeling via DNA reutilization, it remains to be proved that transformation of labeled lymphoid cells into erythrocytic or myelocytic precursors has occurred.

4. Life Cycle of Granulocytes in Man

E.P. Cronkite
P. Stryckmans
A.D. Chanana
T.M. Fliedner
V.P. Bond

Studies are continuing on the use of tritiated thymidine to characterize the life cycle of granulocytes in hematologically normal individuals and in patients with pathologically altered hematopoiesis. Also under study are patients with acute and chronic leukocytic and/or myelocytic leukemia, lymphosarcoma, multiple myeloma, myelofibrosis, pernicious anemia, acute bacterial infection, and nonhematopoietic malignancies, such as breast carcinomas and brain tumors without demonstrable involvement with the blood cell forming tissues. The ap-

pearance of labeled cells in the marrow and their release to the blood continue to be the primary observations that are being made. It has been clearly shown that the release of labeled granulocytes from the bone marrow is a random process and does not follow a "pipeline." In addition, it has been demonstrated that there are two mechanisms by which granulocytes are lost from the blood stream. First there is a random loss from the blood at the epithelial surfaces of the body as demonstrated in oral lavage preparations. In addition, a proportion of the granulocytes undergo a process of senescence and become pyknotic, which takes $\approx$ 24 to 30 hr. This process of senescence truncates the random loss of granulocytes at $\approx$30 hr after their appearance in the peripheral blood. Emphasis is being placed upon the determination of the rate of passage across the myelocyte-metamyelocyte transition and upon studies to determine the DNA synthesis time in myelocytic precursors. Upon establishment of the latter the relative flow rates through the marrow compartment can be established to test the hypothesis that granulocyte production may be "death-controlled."

5. *Estimation of Phases of the Life Cycle of Leukemic Cells in Human Beings by* in Vivo *and* in Vitro *Labeling With Tritiated Pyrimidines*

E.P. Cronkite
T.M. Fliedner
P. Stryckmans
A.D. Chanana
J.S. Robertson

In the continuing studies on leukemic patients, emphasis is at present being placed on *in vitro* labeling of an individual's own cells by tritiated cytidine. Upon autotransfusion, the rates of disappearance of these labeled cells from the peripheral blood and of their appearance in the bone marrow are measured. Six patients with chronic lymphocytic leukemia are under study. Within 24 hr the fraction of labeled cells diminishes by a factor of 2. Thereafter, the intensity of the grain count and the fraction of the small to medium sized lymphocyte cells that are labeled remains constant for a period >3 wk. Thus it appears that in chronic lymphocytic leukemia some of the newly formed cells have life-spans significantly in excess of 3 wk.

Figure 24. Marshallese awaiting examination.

E. DIAGNOSTIC AND THERAPEUTIC STUDIES: SPECIAL STUDIES

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

R.A. Conard
L.M. Meyer
A. Lowrey
A. Watne
R.E. Carter
A. Hicking
I. Lanwai

Annual medical surveys are carried out on the people of the Marshall Islands who were accidentally exposed to fallout ten years ago (March 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 of Rongelap and Utirik Atolls who were involved in the accident are examined, and in addition 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.

Considerable knowledge of the effects of fallout radiation on human beings has been gained from these studies. Gamma radiation with hemopoietic depression was the most serious consequence of the exposure; beta burns of the skin, although partly disabling, were less serious; and internal absorption of radioisotopes appeared to be the least serious of the hazards. Some more specific findings over the past 10 years are as follows: (a) The gamma radiation (highest dose, 175 rads) does not appear to have been related directly to the deaths, illnesses, or diseases that have occurred. (b) The marked depression of peripheral white blood cells and platelet levels that occurred early was followed by rapid increase during the ensuing months, but a slight deficit still persists in the mean levels of these elements compared with levels in the unexposed population. (c) Growth and development studies have shown a slight retardation in the exposed children, particularly in males exposed between 15 and 18 months of age. (d) Studies of late effects, such as leukemia, premature aging, cataracts, and genetic effects, have had largely negative results, although some of the exposed women had an increased incidence of miscarriages and stillbirths during the first few years after exposure. (e) Development of thyroid nodules in three exposed teenage girls (not seen in nonexposed girls) is being investigated. (f) Beta burns of the skin healed within a few months, and in persons with epilation hair regrew by six months. A few skin residua persist in some people in the form of scarring and pigment aberrations

but with no indication of malignant changes. (g) Radioisotopes absorbed internally were rapidly eliminated during the first few years, but since the return of the people to Rongelap (1957) low but detectable levels of Cs^{137}, Zn^{65}, and Sr^{90} have been present in both the exposed and unexposed groups as a result of low-level contamination of the island plant and marine life.

2. *The Effect of the $B^{10}(n,\alpha)Li^7$ Reaction on HeLa Cell Cultures*

J.O. Archambeau
R.M. Drew
J.S. Robertson

The thermal neutron capture reaction of B^{10} is of interest because of the possibility of irradiating cells with high energy particles that have a range in tissue equal to about one cell diameter. In order to compare and evaluate the biological effect of these reaction products, their effect on the proliferative capacity of HeLa cells is being investigated. The technique involves irradiating HeLa cells with thermal neutrons in a boron-containing medium and determining the dose-survival curve. This is shown in Figure 25. The dose-effect curve following $B^{10}(n,\alpha)Li^7$ radiation differs from that obtained following x radiation (Figure 26), the boron curve being straight, while that for x radiation is curved. One mathematical model used to describe these curves is compatible with one thermal neutron capture reaction per cell being sufficient to prevent cell proliferation, whereas for x radiation two or more radiation events are required. Further comparisons of

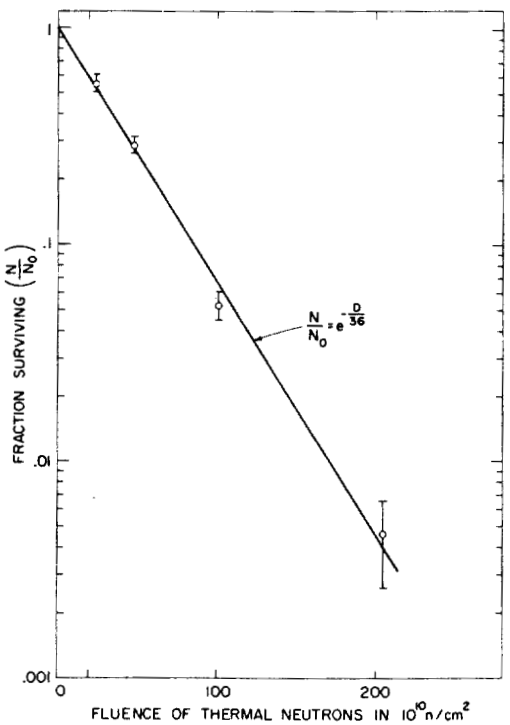


Figure 25. The fraction of 18-hr HeLa cells surviving the thermal neutron capture reaction of B^{10}, represented as an exponential function of dose. (B^{10} : 10 μg/ml medium; averaged values.)

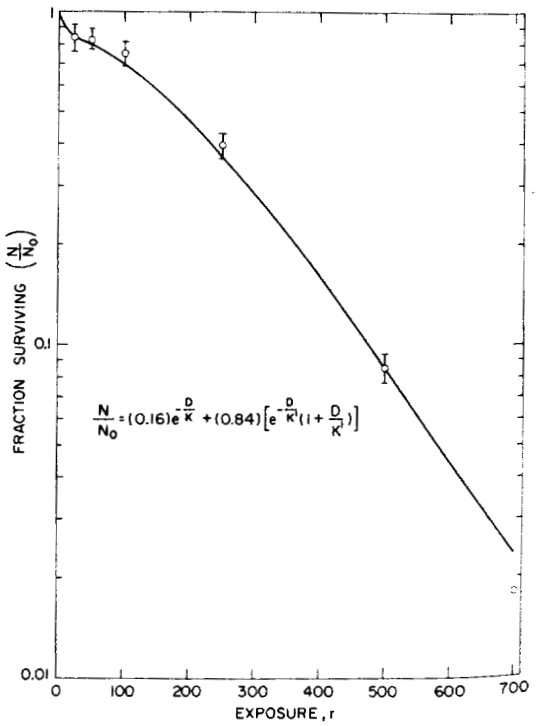


Figure 26. The fraction of 18-hr HeLa cells surviving following x irradiation, shown as a complicated function of dose. (Averaged values.)

the cultures following fractionation of the total dose indicate that radiation damage can be repaired equally well following thermal neutron capture or x radiation.

Comparison studies are in progress to determine the effects of oxygen on survival. In addition, estimates of the numbers and types of abnormal colonies resulting from the two types of radiation are being made.

These studies will be useful in assessing the effect of the $B^{10}(n,\alpha)Li^7$ reaction on animal tissues.

3. Development of an Epithermal Neutron Beam for Possible Use in Neutron Capture Therapy

R. Fairchild
J.S. Robertson

This project is directed primarily toward the development of a dosimetric system capable of evaluating the various components present in a mixed field of reactor radiations. By using the results from the physical dosimetry, the optimal physical conditions can be chosen for the application of the neutron capture process to the therapy of neoplasms. The neutron capture therapy technique involves the localization in a tumor of a capture element, such as B^{10}, Li^6, or U^{235}, which interacts strongly with thermal neutrons reaching the tumor site. A neutron beam, of necessity, exposes some intervening and adjacent normal tissue; the absorbed dose to these normal tissues must be kept at a minimum. The poor penetration in tissue of a thermal neutron beam has previously militated against successful use of the technique. An epithermal beam has been developed which gives increased neutron penetration in tissue, such that the neutron flux density in the clinically interesting region from 0 to 7 cm is always greater than that at the surface. This increased penetration is in contrast to that of the thermal beam, which falls off by a factor of $\approx$16 in 7 cm.

Threshold detectors and tissue-equivalent chambers have been used to evaluate the dose to a small amount of tissue from fast neutrons at both the thermal and epithermal beam ports of the Medical Research Reactor (MRR). Results from the two different methods agree to within 15%. Activation foils and graphite-CO_2 chambers have been used to evaluate the neutron flux density and gamma exposure, respectively. Results of the above measurements have shown that (1) the most favorable conditions for the irradiation of tissue with epithermal neutrons occur with all the D_2O moderator in place, and (2) even with the installation of a cadmium filter in the reactor reflector to produce an epithermal beam, the gamma contamination from the reactor is insignificant.

A system of tissue-equivalent chambers with replaceable electrodes has been developed to evaluate the absorbed dose from fast neu-

trons, thermal neutrons, and gamma rays in any kind of tissue-equivalent phantom. This information is necessary to evaluate the biological results of experiments and to estimate the amount of radiation that can be delivered before the tolerance of normal tissue is exceeded. Typical results are shown in Figures 27 and 28, as measured along the axis of a phantom man at the epithermal beam port. Results of the above measurements and of measurements made at the thermal beam port have shown:

1. The amount of gamma contamination originating from the reactor at both the epithermal and thermal beam ports is relatively low.

2. Assuming the selective presence in tumor of a neutron-capturing isotope at a concentration that would give the same effect as 35 $\mu g/g$ B^{10}, the epithermal beam has the capacity to give a dose to the tumor that is up to 1.6 times the dose to boron-free tissue, at all depths >1 cm in a phantom head.

3. Because the epithermal beam delivers a minimal thermal neutron flux density in the sensitive region of the surface tissue, the addition to normal tissue of ⅙ the tumor B^{10} concentration does not greatly affect the tumor tissue/normal tissue dose ratio.

Experiments are being devised to evaluate the effect of epithermal neutrons on pigskin. A modification of the MRR core is being planned in an effort to increase the epithermal/fast neutron flux ratio seen at the epithermal port.

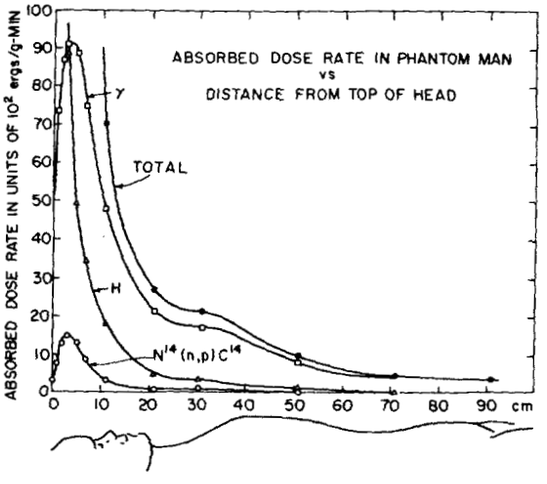


Figure 27. Absorbed dose rate as measured on the long axis of a phantom man at the epithermal beam port of the MRR. Long axis of man coincides with beam axis. Reactor power, 5 MW.

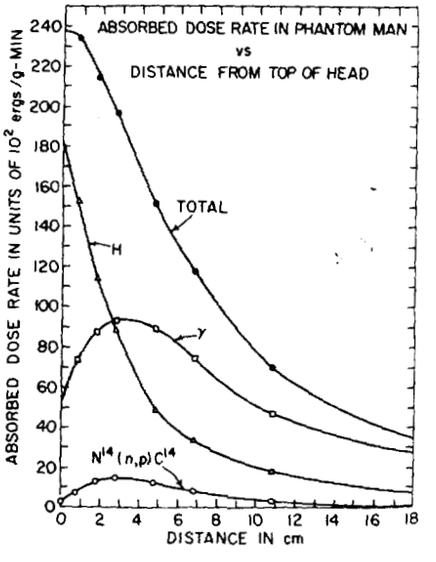


Figure 28. The region from 0 to 18 cm in Figure 27 redrawn to show more detail.

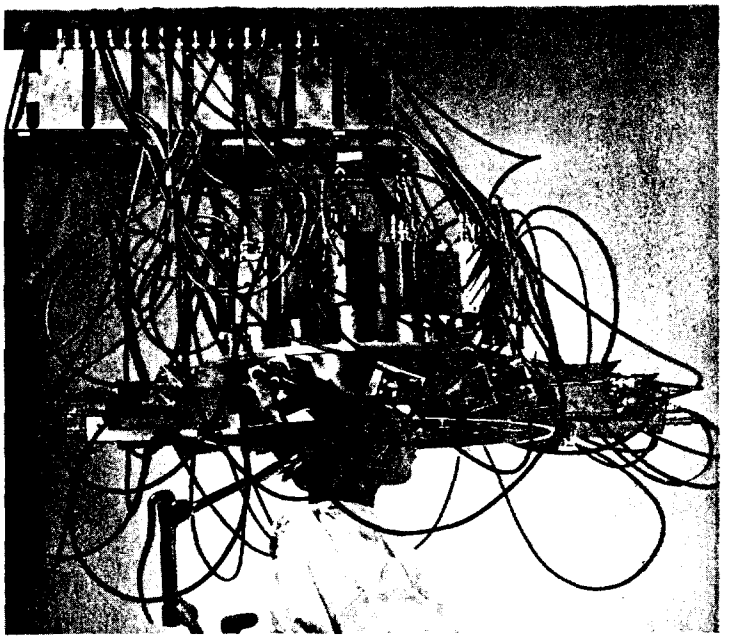


Figure 29. A multidetector positron scanner for locating brain tumors.

4. Positron Scanning

J.S. Robertson
S. Bozzo

A number of compounds that can be labeled with positron-emitting radioisotopes, such as As^{74}, are known to concentrate in certain types of brain tumors more than they do in normal brain. These tumors can be located by the method of positron scanning, which depends upon the principle that within a very short distance from the point of emission a positron is annihilated and two 0.511-MeV gamma rays are emitted in opposite directions. Thus, if a positron-emitting source is placed between two detectors the two gamma rays will be counted simultaneously. In conjunction with coincidence counting techniques, a mapping of the distribution of a positron-emitting radioisotope can be obtained by scanning the head placed between two detectors. In the methods currently in use, it takes up to 40 min to scan the head, and for 3-dimensional localization two scans at 90° to each other are required.

In the expectation that the scanning time could be shortened through the use of multiple detectors, a device using 32 one-inch-diameter NaI crystals in a circular array has been constructed and tested. Instead of restricting the coincidence counting to fixed pairs of crystals, the circuitry has been arranged to record coincidence counts occurring in any of the 178 pairings for which the lines connecting the paired detectors cross the volume of interest.

In principle, such a device should make it possible to deduce the distribution of a positron emitter in the plane of the detectors from counts obtained with the counter in a fixed position. Counting several such planes would give the desired 3-dimensional distribution. In practice, however, unscrambling the data has proved to be a major problem. With the use of the IBM 7094 computer this problem now ap-

pears to be virtually solved. If further tests with sources placed in a phantom head verify the accuracy of the method, clinical trials with patients will be initiated. Although at present a large computer is required for the data reduction, it is expected that the method can eventually be simplified so that the calculations can be achieved with smaller computers.

This project has involved the close cooperation of the Instrumentation Division and the Applied Mathematics Department of the Laboratory.

Figure 30.

5. Computer Applications

J.S. Robertson

Two large, high-speed digital computers, the BNL Merlin and the IBM 7094, are used on a variety of Medical Department problems, and development of further uses is under way. Among the existing uses are the calculation of neutron fluxes from activated gold foil data; spectral "stripping," with data obtained in the whole-body counter; statistical calculations, such as least-squares fits and standard errors; generation of model systems for use in the interpretation of the behavior of radioactive tracers introduced into compartmented systems; calculation of molecular weights from ultracentrifuge patterns; and correlation of information from the multidetector system under development for using positron emitters in the location of brain tumors.

A special computer application arose in the studies of the extracorporeal irradiation of blood, which involves shunting blood from an artery or vein through an x-ray beam and back into a vein. In such a process some portions of the blood recycle and are irradiated many times, while others may completely escape irradiation. The Merlin computer was used to generate curves giving the fraction of the blood that has received a given dose of radiation as a function of the number of cycle times; thus a basis is provided for interpreting the observed effects on the diminution of the white blood cell count.

An entirely different type of computer, an analogue computer, has been developed for the use of the Medical Department by the Instrumentation Division. This instrument is designed specifically for simulating the time course of the behavior of labeled substances introduced into compartmented systems. There is provision for representing data points by generating a set of 96 dots, which are displayed on an oscilloscope simultaneously with up to 4 analogue curves. Fitting the analogue curves to the data by this method in effect solves the differential equations describing the model system used and provides answers in terms of the parameters of the system, such as the sizes of compart-

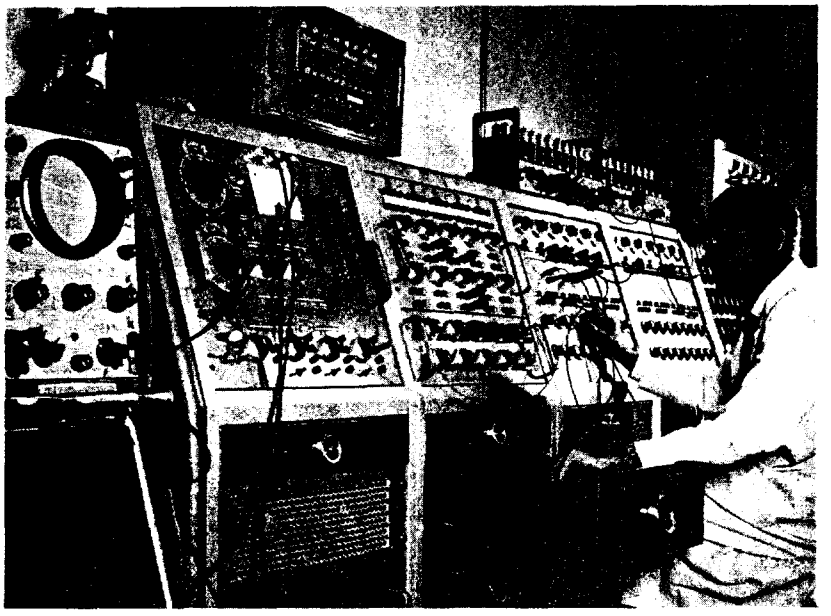


Figure 30. Analogue computer in operation.

ments and the rates of flow of the substance of interest between compartments. This instrument is most useful in the search for suitable models and for obtaining first approximations of the values wanted. For production runs using an established model and for cases in which a least-squares fit is needed, a digital computer program is used.

6. *Technetium-99 m for Scintiscanning*

H.L. Atkins
L.M. Schiffer

The isotope Tc^{99m} has very desirable characteristics for scintillation scanning. The radiation dose is low because of the short physical half-life of 6 hr as well as the virtual absence of significant accompanying beta emission. The gamma emission (99% 0.140 MeV, 1% 0.142 MeV) is detected with high efficiency, since the losses from scattering or tissue absorption are low. With this low energy gamma emitter, collimation and shielding are more efficiently accomplished. The isotope is readily obtained through milking a Mo^{99} generator.

As the pertechnetate the isotope is distributed at first throughout the extracellular fluid and then more slowly enters the intracellular compartment. It is rapidly concentrated in the thyroid, salivary glands, and stomach, and the concentrations in the thyroid and salivary glands are quite suitable for scanning. It is useful also for brain scanning and, as a colloid in combination with sulfur, can be used for liver and lung scanning.

E. DIAGNOSTIC AND THERAPEUTIC STUDIES

7. *Factors Affecting Radiation-Suppressed Hepatic Uptake of Colloidal Gold*

H.L. Atkins

A study has been conducted based on observations made elsewhere that in the case of patients undergoing radiotherapy for hepatic metastases, there appears to be a reduction in uptake of colloidal radiogold in an area corresponding to the outline of the treatment field. It is thought that radiation probably has a direct effect on the phagocytes in the radiation field resulting in death of the cells rather than in suppression of the immune mechanism. Using I^{131}-tagged rose bengal and colloidal radiogold, studies are being initiated on the effect of both local and whole-organ radiation and of immunosuppressive drugs on the hepatic scan and blood flow.

8. *Neutron Radiography*

H.L. Atkins

Neutron radiography differs from x or gamma radiography in that absorption is dependent on the presence of specific nuclides rather than on the density of electrons. Materials that may be dense to x rays, such as lead or steel, are rather transparent to neutrons, while such x-radiolucent materials as Lucite or fats are opaque to neutrons because of the high content of hydrogen.

Biological materials are suitable subjects for neutron radiography because of the high hydrogen content in the cell water as well as the presence of fatty substances in various organs, especially the brain. Contrast materials utilizing high capture cross-section nuclides are potentially useful for visualizing certain structures.

Technical problems exist in applying the method because of the great amount of scattering by hydrogen. Approaches being used to counteract the subsequent image degradation are (a) the use of a grid similar in type to that used in x radiography, and (b) exploitation of the epithermal beam.

9. *Cytogenetic Studies*

R.A. Conard

In recent years improved techniques of cell culture, particularly the preparation of chromosomes for satisfactory visualizaton, have given strong impetus to cytological investigations. Among such investigations, the effects of ionizing radiation in producing alterations in numbers and aberrations of chromosomes have been clearly shown in animals. Similar effects in human beings exposed to radiation have been reported by Bender and Gooch in the Y12 cases at Oak Ridge, and by Court-Brown in spondylitics treated with x rays. The Marshallese population exposed to various doses of whole-body radiation and

skin burns from accidental fallout is being observed for the presence of such cytogenetic effects. Peripheral blood and skin cultures from irradiated individuals are being carried out at Rongelap Island, and the samples brought to this Laboratory for final preparation and study. Initial observations indicate that certain aberrations are present. In concurrent animal studies, attempts are being made to culture rat blood and skin to evaluate radiation effects in this species.

F. 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 other forms of cancer, diabetes mellitus, and essential hypertension.

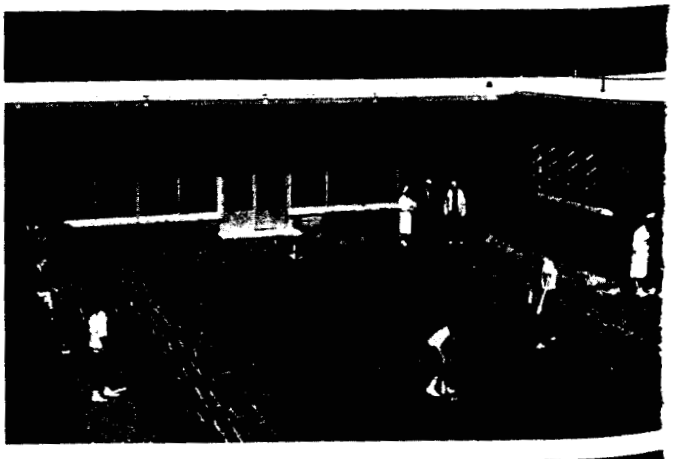
Patients are admitted only upon referral by their physicians or clinics and are returned to the care of their physician at the end of their hospitalization. The great majority of patients are residents of Suffolk and Nassau Counties on Long Island, but many are referred from New York City and other parts 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. 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.

Patients using some of the equipment and materials available for occupational and physical therapy.



Patients relaxing in courtyard.

The Medical Research Center

Although all the special facilities of the Laboratory are available to the scientists of the Medical Department, most research activities of the Department are carried on in the Medical Research Center. Dedicated December 16, 1958, "to the abatement of man's ills through the application of knowledge of nuclear physics to medicine," the new building has proved particularly suited to the research program.

The design criteria for this building were based on three objectives: (1) the rendering of improved professional services to the patient in accordance with the precepts of modern scientific medical usage, thereby assuring the best possible care of the ill. (2) The provision of services and facilities inexpensively adaptable to the changing requirements of scientists engaged in fundamental research. (3) Economical maintenance. The unorthodox design of the building resulted from attempts to meet the functional requirements of the various activities of the Medical Department under a single roof.

The building contains the Hospital, Industrial Medicine Clinic, Laboratory Wing, Central Administration Service Area, and the Medical Research Reactor. Oriented to the maximum advantage of the terrain and weather conditions, the Hospital is at the west end of the building; it has a 44,000-ft^2 area and a capacity of 48 beds. Each of 4 medical nursing units or pavilions consists of 12 individual patient rooms on the periphery of a 72-ft circle, in the center of which is the nurses' station. This arrangement minimizes the amount of walking by the nurses in the course of their duties, and makes each room visible from the nurses' station.

Although the hospital does not offer surgical service, a completely equipped emergency operation unit is located in the ancillary service area. The x-ray suite contains two machines for diagnostic x rays, one of which has fluorographic attachments including an 8-in. image amplifier for minimizing patient exposure. Pharmacy, central sterile supply, occupational therapy suite, physical therapy gymnasium, medical photography room, visitors' waiting room, dressing and locker rooms, and a dietary wing complete the hospital service areas.

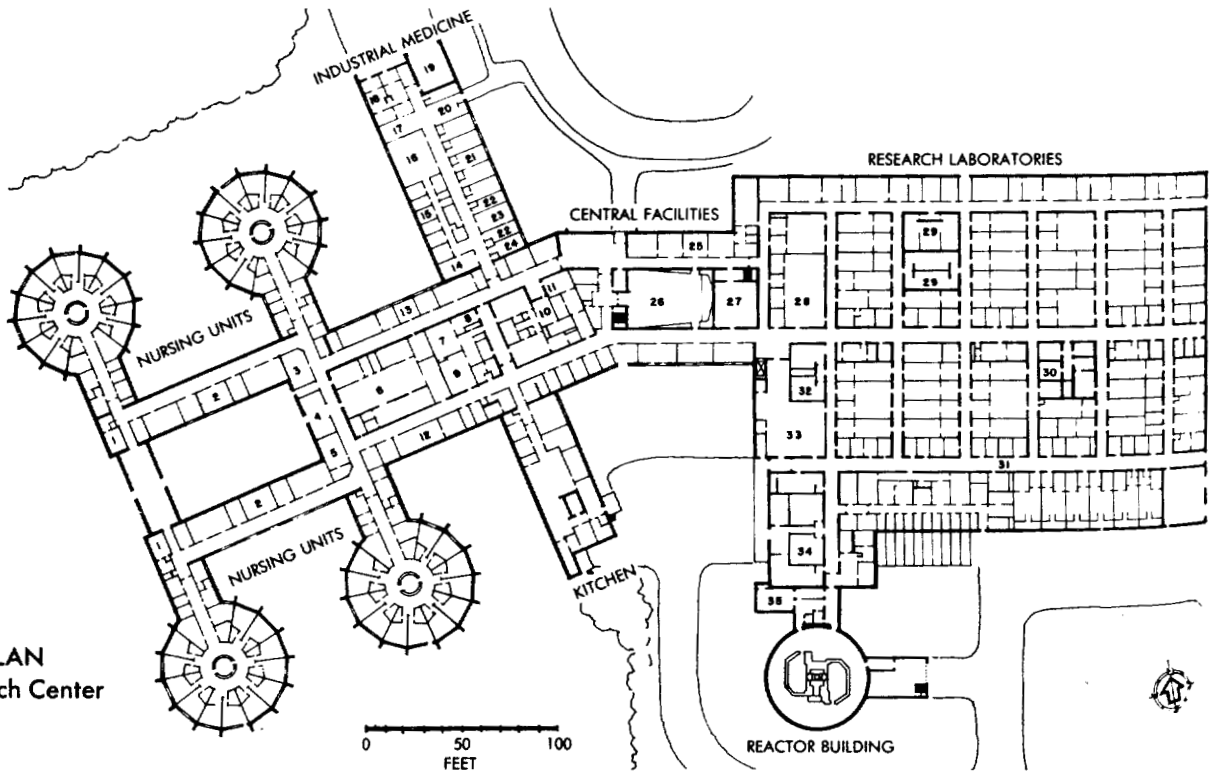
At the Industrial Medicine Clinic all employees receive pre-employment, termination, and annual physical examinations. Emergency first aid is also provided. A unique and particularly important feature of this wing is the decontamination suite, used only for treating persons contaminated by radioactive material. The suite contains two identical units of three rooms, one for immediate showering and scrubbing of the skin, another for examination and first-aid treatment, and a radioactively "clean" rest area where the patient may await further processing or discharge.

The central area of the Center contains a seminar room with a seating capacity of 130 and a medical library which now contains 4301 books and 4417 bound volumes of journals.

The laboratory section of the Medical Research Center building provides an area of 58,000 ft^2 on the main floor for laboratories, offices, and special service facilities to ac-

commodate the full-time staff and a number of visiting scientists and research collaborators. A basement under this section provides space for the mechanical and electrical ducts and connections. The layout of these rooms and the final selection of a basic module of 11×11 ft resulted from eight years of experience in modifying the temporary structures previously used by the Medical Department to meet the continually changing needs of the scientific staff.

Available for scheduled use by each scientist are many departmental facilities such as controlled temperature rooms with wide ranges of temperature and humidity; cold rooms; a subzero room; 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;



FLOOR PLAN
Medical Research Center

LEGEND

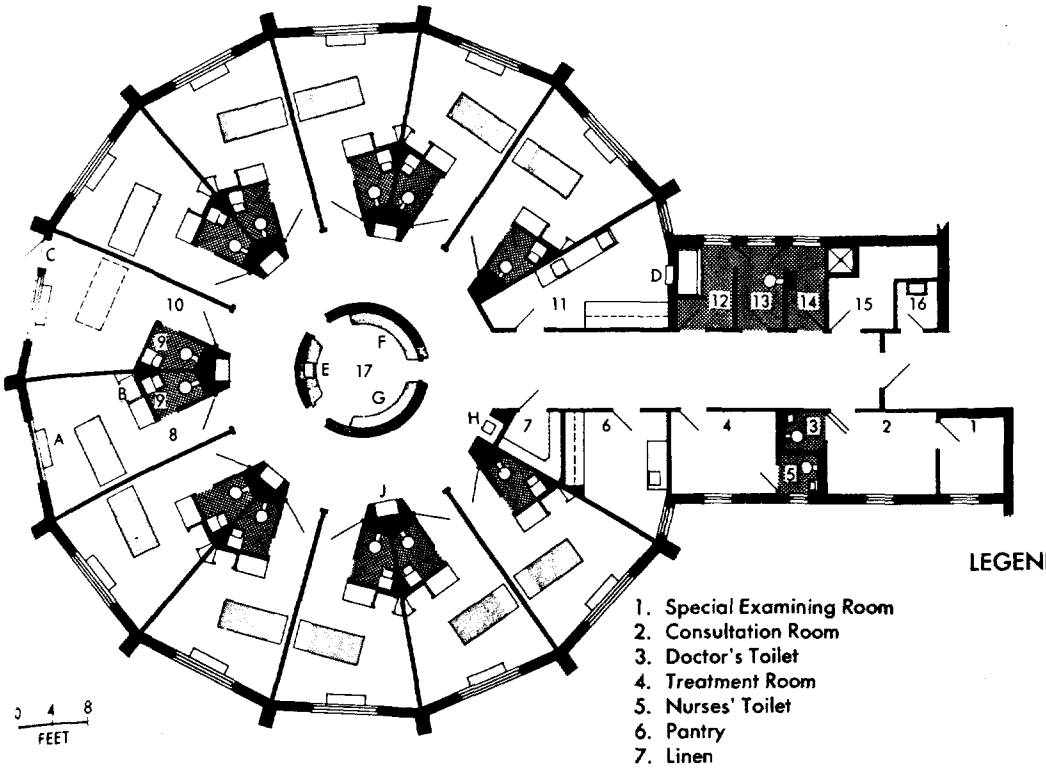
RESEARCH HOSPITAL
1. Resident Quarters
2. Day Rooms, Visiting Rooms
3. Hospital Waiting Room
4. Patients' Library
5. Medical Photography
6. Occupational and Physical Therapy
7. Emergency Operating Room
8. Anaesthesia Room
9. Central Sterile Supply
10. X-Ray Suite
11. Pharmacy Suite
12. Hospital Staff Locker Rooms
13. Hospital Offices

INDUSTRIAL MEDICINE
14. Hematology Laboratory
15. Special Testing Rooms
16. Treatment Room
17. Clerical Station
18. Decontamination Suite
19. Ambulance Garage
20. Lobby
21. Examining Rooms
22. Outpatients' Toilet
23. Urinalysis Laboratory
24. Dishwashing Room

CENTRAL FACILITIES
25. Administrative Offices
26. Seminar and Conference Room
27. Medical Library

LABORATORIES
28. Clinical Chemistry Laboratory
29. Sample Counting Rooms
30. Special Temperature Rooms
31. Animal Quarters
32. *In Vivo* Counting Rooms
33. Stockroom
34. Isotope Receiving, Preparation, Administration

REACTOR BUILDING
35. Patient Preparation and Operating Suite



TYPICAL NURSING UNIT

LEGEND

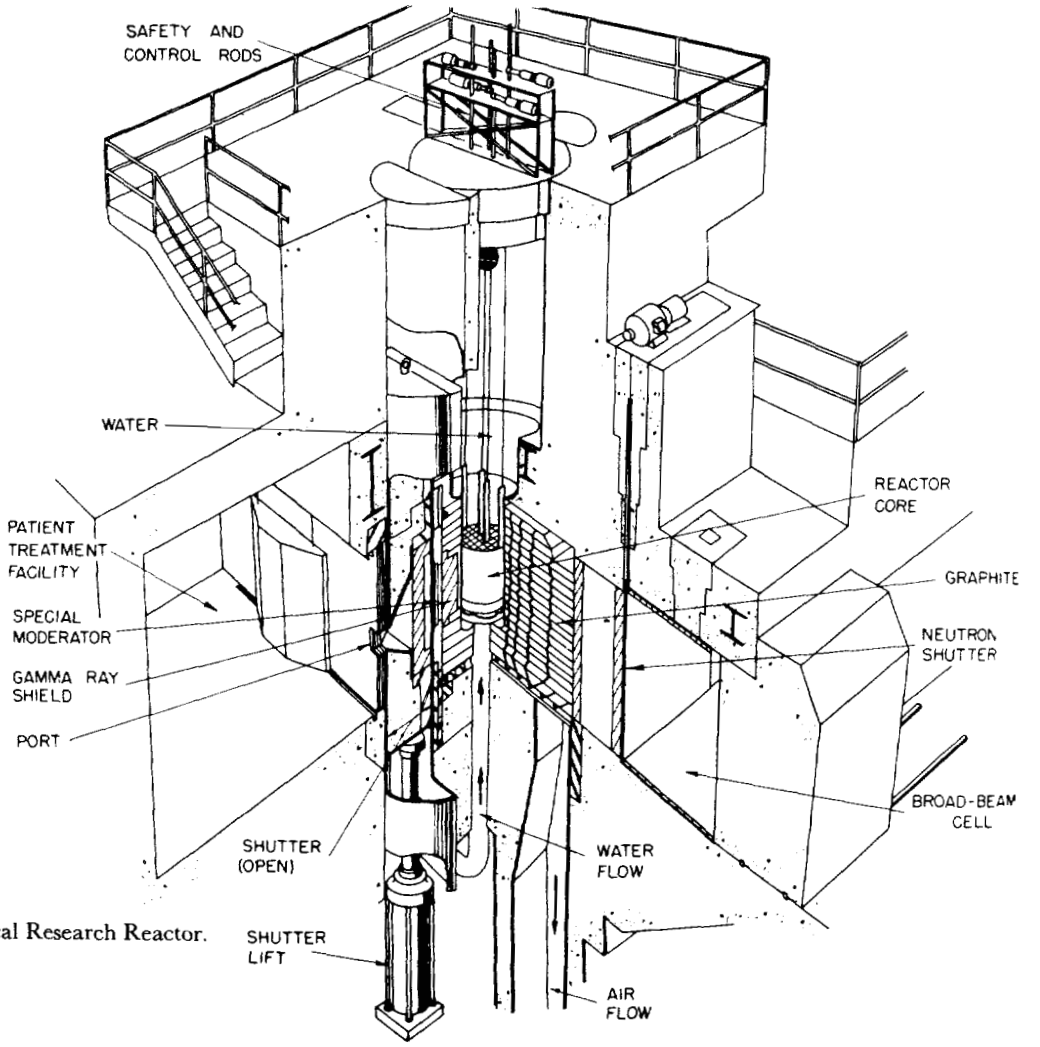
1. Special Examining Room
2. Consultation Room
3. Doctor's Toilet
4. Treatment Room
5. Nurses' Toilet
6. Pantry
7. Linen
8. Patient's Bedroom (typical)
   - A. Heating, ventilating, air conditioning unit
   - B. Dresser-wardrobe unit
9. Patient's Toilet (typical)
0. Patient's Bedroom (usable as Dayroom)
   - C. Emergency exit
11. Utility Room
    - D. Bed-pan sterilizer
12. Patients' Bathroom
13. Toilet (wheel-chair patients)
14. Patients' Shower Room
15. Storage Room
16. Janitor's Closet
17. Nurses' Station
    - E. Medicine section
    - F. Chart desk
    - G. Clerical desk
    - H. Drinking fountain
    - J. Storage closet (typical)

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 too active for general laboratory use.

The Medical Research Reactor (MRR) has been built as an integral part of the laboratory and hospital in Brookhaven's Medical Research Center. This reactor was planned jointly by staff members of the Medical Department and scientific personnel from the Nuclear Engineering Department and Reactor Operations Division. It was constructed for the purpose of exploring the possible applications of nuclear reactors in the study of man and the diseases of man.

The design criteria of the reactor include: (1) flexibility of the core and reflector to provide the required quantity and quality of radiations in desirably short time intervals; (2) shielding, shutters, and other radiation control elements for adequate delivery and limitation of radiation fields in the treatment vaults; and (3) provision of isotope production tubes for the instant use of short-lived radioactive materials *in vivo* or for activation analysis of biological materials.

The MRR had originally a nominal design power level of 1 MW for continuous operation. Subsequent to the initial testing program, the AEC Reactor Safeguards Committee granted permission for routine operation at levels as high as 3 MW. After a year of experimental use, permission was granted to operate at 5 MW for periods as long as 10



Cutaway diagram of the Medical Research Reactor.

min., when especially scheduled. The limitation on excess reactivity was raised from 1.0% to 3.0%; typical operation is at 1.85%. The enriched U^{235} used in 18 fuel elements totals $\approx$2.52 kg. The core is cooled by natural water. Neutron moderation in the core is provided by the water and by graphite fillers. The moderator and reflector extend beyond the core container as an air-cooled graphite layer one yard thick. In the direction of neutron flow toward the treatment vaults the reflector is fitted with special sections to control radiation quality. To reach the irradiation port, the neutrons must pass through a succession of graphite, heavy water, and bismuth sections, in part surrounded by thick plastic reflector surfaces, with an outer boundary of boral and heavy concrete to prevent radiation leakage into protected areas.

Shutters were built into the neutron apertures leading to the treatment vaults for experimental irradiation procedures. Each shutter incorporates flexible beam-directing and neutron-moderating arrangements in a hydraulically operated device weighing some 20 tons with an effective opening interval of $\approx$3 sec. Two irradiation rooms were provided. Since these rooms are enclosed within shielding walls and are equipped with neutron activation tubes passing directly to the reactor core, they may also be employed to investigate the diagnostic and therapeutic possibilities of radioactive isotopes of very short half-

life. Preparation for either of these purposes can thus be carried out without interference from other reactor activities and under completely standard hospital conditions.

Another special exposure room, built inside the reactor shielding against a thermal column, forms a cell ($\approx$5 ft in each dimension) in which objects can be subjected to the complete gamut of reactor radiations. By use of selective shielding curtains and radiation converter plates, it is possible to expose living systems to the various component radiations separately.

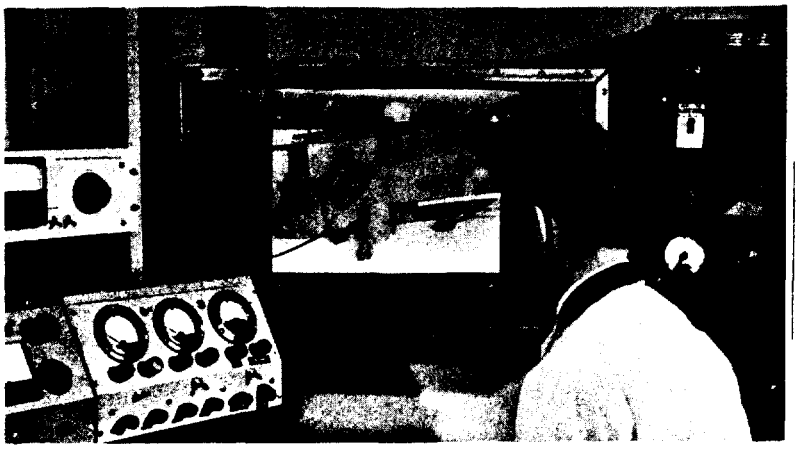
Exposure holes and tubes leading inside the reactor make possible investigation of such matters as the induction of radiation effects in biological materials and the tagging of biological materials by the neutron activation principle or by radiation catalysis. Certain of these experimental sites are amenable to research on the physical and geometrical factors that control the penetration of the several radiations and their patterns of energy deposition in tissues.

A whole-body counting system has been in operation at BNL since May 1959. The design was based on a "portable" prototype built in 1958 to permit direct measurement of internally deposited gamma-emitting fission products in the Marshallese people exposed to radioactive fallout from the March 1954 nuclear weapons test. This field instrument was based on the system designed by C.E. Miller and built at Argonne National Laboratory.

The whole-body counting system is designed for detecting very low concentrations of internally deposited gamma-emitting material and is built around a scintillation spectrometer. The counter utilizes a NaI (Tl-activated) 4×8-in. crystal and is connected to a 100-channel pulse height analyzer. The crystal detector is located in a 6×7×9-ft shielded room constructed of 6-in. steel and lined with lead, cadmium, and copper for shielding against the low energy components of background radiation. The heavy shielding effectively reduces the gamma radiation to the detector from both cosmic rays and building materials and increases the signal-to-noise ratio for the system.

The Medical Research Center has recently acquired two new concrete block buildings to house experimental animals. One of these, designed as a quarantine and isolation building, has 2900 ft^2 of floor space and includes 10 individually air-conditioned rooms to house animals. Its principal purpose is to provide temporary holding facilities for observation of animals newly acquired for research as a safeguard against the introduction of infectious diseases.

The second building, designed to house large animals such as cattle and swine to be used in research, has 3600 ft^2 devoted to animal quarters and laboratory space used principally for animals undergoing extracorporeal irradiation of their circulating blood. The area also houses the Co^{60} fluid irradiator source.



Animal being irradiated with neutrons at the Medical Research Reactor as part of a study to determine the effects of radiation on living tissues.

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(Addenda since July 1, 1963)

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