ACRH-103

ARGONNE CANCER RESEARCH HOSPITAL
950 EAST FIFTY-NINTH STREET • CHICAGO • ILLINOIS 60637

Meeting of the Advisory Committee to the Argonne Cancer Research Hospital Program

MARCH 4–5, 1965

LEON O. JACOBSON, M.D.
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F O R E W O R D

In 1948 the U. S. Atomic Energy Commission approved the establishment of a cancer research hospital with appropriate laboratory facilities at the University of Chicago. It was intended that this hospital be administered by the Medical School and Clinics of the University, and that its facilities be available to qualified investigators. After nearly three and one-half years of building, the first patient was admitted on January 10, 1953, and the formal opening date of the hospital was March 10, 1953.

The purpose and program of the hospital are directed toward the exploitation of high energy sources for the treatment of malignancies, the study of the biological effects of radiation, the use of radioisotopes as tracers in the study of normal and disease states, and in the diagnosis and therapy of disease. The scientific program is correlated in general with that of the Division of the Biological Sciences and the University of Chicago Hospital and Clinics, of which the Argonne Cancer Research Hospital is a part. Close relations are also maintained with the Argonne National Laboratory at Argonne, Illinois.

From the beginning the staff of the ACRH has encouraged participation in its research program by graduate and undergraduate medical students and advanced students in the biological sciences at the University of Chicago. It has also taken an active part in various research investigations of general interest with University faculty members in the Life and Physical Sciences. This interdisciplinary effort has proved of great value to the ACRH program. Student participation and faculty collaboration have also made possible the training of large numbers of undergraduate and graduate students (as well as faculty) in the use of radioisotopes in research, diagnosis and treatment of various disease states.

Argonne Cancer Research Hospital has eight floors, with a total area of 102,500 square feet. Two floors with 56 beds are devoted to clinical research. The remaining six floors house high energy radiation equipment, electronic and machine shops, an animal farm, and conventional research laboratories. The staff is composed of 55 scientists, 160 technicians, nurses, and non-technical laboratory personnel, many of whom are paid in part by the University. Since the University clinical departments assume care of the patients at the ACRH, any part of a staff member's time devoted to professional care as distinguished from research is paid for by the University and does not feature in the ACRH budget. This accounts

for the fact that the scientific staff totals 55½ while the actual number of scientific man years devoted to the research program is 45.

H. Stanley Bennett, M.D.
Dean
Division of the Biological Sciences
University of Chicago

ADVISORY COMMITTEE TO THE

ARGONNE CANCER RESEARCH HOSPITAL PROGRAM

FIRST MEETING: MARCH 4 - 5, 1965

MEMBERS OF THE COMMITTEE

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Chief of Medical Staff Harvard Medical School
Massachusetts General Hospital
Boston, Massachusetts

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Professor and Executive
Department of Radiology
Stanford University School of Medicine
Palo Alto, California

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School of Medicine
University of Washington
Seattle, Washington

Dr. Joseph F. Ross
Director
Laboratory of Nuclear Medicine and Radiation Biology
University of California School of Medicine
Los Angeles, California

PROGRAM*

Thursday, March 4, 1965 . . . CHAIRMAN, Dr. L. O. Jacobson

Morning Session	Immunology and Molecular Biology
9:00	Introductory Remarks H. Stanley Bennett Dean of Biological Sciences, University of Chicago
9:05	The Influence of Total-Body Irradiation and Other Factors on the Fate of Particulate Antigens and on the Migration of Antibody Forming Cells R. W. Wissler (pp. 1 - 5)
	Studies on the Destruction of Human Red Cells by the Complement System S. Yachnin (pp. 23 - 27)
	Some Biochemical Studies of Red Cell Differentiation E. Goldwasser (pp. 28 -32)
10:35	INTERMISSION
10:50	Protein Synthesis in Heart Muscle M. Rabinowitz (pp. 32 - 33)
	An Effect of Polycyclic Aromatic Hydrocarbons on Bacteriophage Development S. B. Weiss (pp. 34 - 36)
12:05	LUNCH
Afternoon Session	Experimental and Clinical Studies of Cell Differentiation
1:30	The Riddle of Polycythemia Vera C. W. Gurney (pp. 37 - 40)

*Page numbers locate abstracts and lists of senior authors and co-authors.

Afternoon Session (continued)

The Mechanism of the Testosterone Effect on Erythropoiesis
W. Fried (pp. 41 - 42)

A Lesson From an Anemic Mouse
A. Kales (pp. 43 - 44)

Chromosome Abnormalities in Patients with Hematological Abnormalities
J. Rowley (pp. 44 - 47)

3:00 INTERMISSION

3:15 General Metabolic Studies

Selected Aspects of Uric Acid Metabolism in Man
L. B. Sorensen (pp. 62 - 64)

Effects of Estrogens on Hepatic Excretory Activity
A. Kappas (pp. 65 - 67)

Intermediary Metabolism of Irradiated Rats
G. V. LeRoy (pp. 68 - 70)

6:00 DINNER for visitors and participants at the Quadrangle Club, 1155 East 57th Street

Friday, March 5, 1965 . . . . CHAIRMAN, Dr. P. V. Harper, Jr.

Morning Session — Problems in Scanning

9:00 Theoretical Considerations
R. N. Beck (pp. 76 - 79)

Instrumental Design and Construction
D. B. Charleston (pp. 79 - 101)

Chemical and Biological Aspects
P. V. Harper, Jr. (pp. 101 - 108)

Morning Session (continued)

10:30	INTERMISSION
10:45	Radiation Effects
	Neurological Studies in Monkeys Following Thalamic Lesions with ^{90}Y S. Schulman (pp. 109 - 111)
	Clinical Applications of Beta Sources in Neurosurgery J. F. Mullan (pp. 112 - 114)
	The Late Effects of the Deposition of Radium in Man R. J. Hasterlik (pp. 116 - 119)
12:00	LUNCH

Afternoon Session

1:30	Tour of Argonne Cancer Research Hospital
2:00	Linear Electron Accelerator (demonstration in sub-basement) L. S. Skaggs (pp. 126 - 129)
2:30	Executive Session

ARGONNE CANCER RESEARCH HOSPITAL

H. Stanley Bennett	Dean, Division of Biological Sciences, and Professor of Anatomy, University of Chicago
Leon O. Jacobson	Director, Argonne Cancer Research Hospital, and Professor of Medicine, Chairman of the Department of Medicine, University of Chicago
Paul V. Harper	Associate Director, Argonne Cancer Research Hospital, and Professor of Surgery, University of Chicago
C. William Kupferberg	Assistant Director for Administration, Argonne Cancer Research Hospital, and Executive Assistant, Department of Medicine, University of Chicago

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	Scientific Staff	Departmental Affiliation University of Chicago
Robert N. Beck	Research Associate (Asst. Prof.)	Medicine
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James C. Bland	Assistant	
James W. J. Carpender	Professor	Radiology
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Louis A. DeSalle	Associate Scientist	
Margot Doyle	Senior Scientist	
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Scientific Staff (continued)

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Eric L. Simmons	Research Associate (Assoc. Prof.)	Medicine
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Leif B. Sorensen	Assistant Professor	Medicine
Alvin R. Tarlov	Assistant Professor	Medicine
Samuel B. Weiss	Professor	Biochemistry
Robert W. Wissler	Professor, Chairman of Department	Pathology
Stanley Yachnin	Assistant Professor Markle Scholar	Medicine
Lawrence T. Zimmer	Associate Scientist	

Collaborating Personnel at the University of Chicago

Donald Cannon	Instructor	Pathology
Paul E. Carson	Assistant Professor	Medicine
Jerry G. Chutkow	Instructor	Medicine
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Collaborating Personnel at the University of Chicago (continued)

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Katti Dzoga	Junior Scientist	Pathology
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	Director, Health Division	Health Division, Argonne National Laboratory
Walter Fried	Post-Doctoral Fellow (Asst. Prof.)	Medicine
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Peter Lazarovitz	Resident (2nd year)	Radiology
Charles Miller	Associate Scientist	Argonne National Laboratory
John F. Mullan	Professor	Surgery
Daniel Paloyan	Intern	Surgery
Edward Paloyan	Instructor and Senior Resident	Surgery
Gary Pick	Resident (2nd year)	Dermatology
Jerome Petasnick	Resident (2nd year)	Radiology
Donald Rowley	Associate Professor	Pathology
John H. Rust	Professor	Pharmacology (Section of Nuclear Medicine)
Sidney Schulman	Associate Professor	Medicine

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Nels M. Strandjord	Associate Professor	Radiology
Francis Straus	Instructor	Pathology
Radovan Zak	Assistant Instructor	Medicine Biochemistry

Student Research Associates

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Michael Axelrad	Post-Doctoral Trainee and Ph.D. Candidate	Pathology
Maurice Barcos	Graduate Student (1)	Biophysics
Howard Benensohn	Medical Student (3) and M.S. Candidate	Pathology
Donald Cantway	Medical Student (2) and M.S. Candidate	Pathology
Albert Dahlberg	Graduate Student (4)	Biochemistry and Medicine
Keith Dixon	Graduate Student (1)	Radiology
Martin Gross	Graduate Student (1)	Biochemistry and Medicine
Richard Gumport	Graduate Student (2)	Biochemistry
Michael Hrinda	Graduate Student (2)	Biochemistry
Robert Hunter	Medical Student (4) and Ph.D. Candidate	Pathology
Arthur Kales	Medical Student Asst. (4)	Medicine
John Kurnick	Medical Student (3)	Medicine
Irving Lerch	Graduate Student (2)	Radiology

Student Research Associates (continued)

Bruce Merchant	Post M.D. Trainee and Ph.D. Candidate	Pathology
John W. Moohr	Medical Student (4)	Medicine (1 year Biochemistry)
Nehe Nwankwo	Medical Student (2) and M.S. Candidate	Pathology
Robert Okin	Medical Student (2) and M.S. Candidate	Pathology
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Douglas White	Medical Student Asst. (2)	Medicine

ABSTRACTS

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PROBLEMS IN SCANNING

Page

STUDIES WITH HIGH ENERGY RADIATIONS

STUDIES IN IMMUNOLOGY

Studies in Host-Tumor Balance

R. W. Wissler, K. Dzoga, F. Straus, H. Benensohn

and K. Craft

Recent evidence obtained in this laboratory has identified endogenous serum protein fractions in normal humans and cancer patients which have a marked cytotoxic action on established human cancer cell lines. Some of these require complement for their activity and some do not. Attempts are now being made to purify and identify these substances by means of column chromatography (DEAE), Sephadex chromatography, immunoelectrophoresis, thin layer chromatography, etc. In addition to their complement requirements, these substances seem to vary substantially in concentration and activity in the sera of cancer patients (as compared to normal patients), and in the sera of cancer patients before and after removal of the primary neoplasm. The factors controlling these cancer related fluctuations are therefore being studied in detail.

Three new recently-reported methods of quantitating cancer cell cytotoxic activity in human serum fractions are being compared with each other and with well defined proteolytic esterase systems in order to arrive at better ways of evaluating this important function, which is likely to influence host-tumor balance.

Experiments with cancer cells from recently removed surgical specimens, serum of the corresponding cancer patients, and

lymphocytes from their regional lymph nodes (along with suitable control specimens) are now being initiated. These extensions of the previous studies are designed to furnish further information which may be correlated with the host-tumor balance of forces in the individual patient.

Studies of the factors controlling the cancer localization of fibrinogen and antibody to fibrinogen in the tumor bearing host are being continued in order to obtain more information on the ultrastructural site of localization and the factors controlling the presence or absence of localization in various kinds of tumors.

New Approaches to the Study of Tumor Immunity and Tumor Specific Antigens

B. Merchant, F. Tweet, N. Nwankwo, and R. W. Wissler

Highly avid and specific antibodies have been produced against unique antigens of the Ehrlich ascites tumor cell. Rabbits received passively administered homologous anti-normal mouse tissue antibody prior to active immunization with preparations of Ehrlich tumor cells or cell fractions. This procedure, applied to tumor antigens for the first time in these current studies, has been termed "passive-active immunization." The antibodies thus prepared have been termed "passive transfer modified" antibodies.

These antibodies demonstrate highly selective localization on, and avidity for, Ehrlich tumor cells and a very low cross-reactivity with normal CP-1 mouse tissues. This fact has been demonstrated directly with isotope-labeled antibody localization studies both in vitro and in vivo. Corroboration of this fact has been accomplished with the immunofluorescent technique. Gel-diffusion studies show minimal or absent reactivity of these sera with soluble tumor components, and have also served to rule out the presence of detectable cross-reactions with any soluble components or products or normal tissues.

Comparison of toxicities for conventional and passive transfer modified antibodies has provided definite evidence that use of "passive-active immunization" results in the production of antibodies with no detectable cytotoxicity for circulating mouse erythrocytes. General toxicity is also quite low, since no deaths resulted when a dosage was employed equal to the LD_{50} dosage for a standard preparation of anti-Ehrlich cell antibody. In addition, a single injection of passive transfer modified antibody has resulted in increased survival of CF No. 1 mice bearing well-established ten-day subcutaneous Ehrlich tumors.

The technique of "passive-active immunization" permits production of specific and potent antibodies against selected components of complex antigenic mixtures.

This approach is now being extended to the study of immune sera against methylcholanthrene-induced breast carcinomas in inbred rats. Lines of these cancer cells have been successfully established in tissue culture and are being used for antigenic stimulation.

Furthermore, this line of approach is also being used to prepare and to study antibodies against the so-called "minimum-deviation" hepatomas such as the Morris #5123.

The Influence of Total-Body Irradiation and Other Factors on the Fate of Particulate Antigens and on the Migration of Antibody Forming Cells

D. Cannon, R. Hunter, and R. W. Wissler

In recently immunized animals, total-body irradiation with spleen shielding is followed by a massive redistribution of viable lymphocytes. The thymus does not participate as a recipient in this recirculating lymphocyte pool.

Circulating lymphoid cells which repopulate the irradiated spleen (with body shielding) are able to restore its ability to exhibit immune red pulp hyperplasia and to produce circulating agglutinins.

A very large proportion of the cells of the red pulp of the rat spleen are labeled by a single small dose of tritium thymidine administered 2 or 3 days after intravenous particulate

antigen injection and 18 hours after total-body irradiation with spleen shielding. Subsequent radioautographs reveal that some of the large pyroninophilic cells of the red pulp (as well as the cells resembling lymphocytes which differentiate from these large cells beginning between the third and fourth day after antigen injection) enter the blood stream, migrate to the lymph nodes and bone marrow, and return thence to the splenic follicles. This migration may be important in the dissemination of immunological information.

Recent experiments on irradiated animals (with and without spleen shielding) as well as on very young, recently immunized animals have indicated two major patterns of phagocytic activity in the rat spleen. Antigenic materials (^{125}I-labeled typhoid flagella) localize to a much greater extent and after a slight lag phase, in the follicles of the spleen as compared to inert substances like titanium dioxide, which are likely to remain largely in the red pulp including the marginal zone. This migration of labeled antigenic substances from red pulp to follicle appears to be strongly correlated with the restoration of the antibody-forming capacity after x-ray or with its postnatal development.

Immune Mechanism in Tumor Rejection

E. L. Simmons

Many mouse tumors are highly strain specific and can be transplanted only to the strain of origin, its hybrid offspring, or to other lines within the same histocompatibility gene-II group. Other tumors, on the other hand, are non-specific. Thus, Ehrlich's ascites tumor (EAT), when injected intraperitoneally, "takes," and kills all strains of mice. Apparently the host body is not completely passive immunologically to such invading cells, but succumbs to their rapid invasiveness, since inoculation of EAT subcutaneously can be conquered with varying degrees of success depending upon the strain of mouse.

We have given massive doses of X irradiation to EAT cells to determine the killing dose, and have transplanted such cells intraperitoneally or subcutaneously into normal mice and also into hosts whose immune mechanism had been suppressed by 500 R. A dose of 4,000 R to EAT cells was required to prevent their take intraperitoneally into normal mice. However, intraperitoneal injection of such cells killed mice weakened with 500 R, and massive doses of irradiation of the order of 100,000 R were required to render EAT cells completely harmless to weakened mice (Table 1).

It is apparently easier immunologically for mice to suppress a subcutaneous invasion of EAT cells than an intraperitoneal

Table 1. Number of Mice Surviving EAT cells (Per Cent)

X ray dose to EAT cells	Implanted Intraperitoneally		Implanted Subcutaneously	
	To normal mice %	To 500 R weakened mice %	To normal mice %	To 500 R weakened mice %
0 R	0	0	CF No. 1 - 80 C3H - 60 C57BL/6 - 40 $B6D2F_1$ - 60	0
10,000 R	100	0	100	CF No. 1 - 0 C3H - 0 C57BL/6 - 60 $B6D2F_1$ - 0
20,000 R	100	0	100	CF No. 1 - 0 C3H - 0 C57BL/6 - 80 $B6D2F_1$ - 40
50,000 R	100	0	100	CF No.1 - 100
100,000 R	100	20	100	CF No.1 - 100
150,000 R	100	100	100	100

injection, but in both cases it was observed that EAT cells, despite massive doses of irradiation, formed solid tumors and killed host mice weakened with 500 R. The C57BL/6 mouse strain was more resistant than CF No. 1 or C3H. The $B6D2F_1$ hybrid, a cross of the C57BL/6 with DBA/2, although larger than its parent in body size, was less potent

immunologically and succumbed to EAT implants that the C57BL/6 parent was able to withstand.

Thus different parts of the body can function at different levels of competence in suppressing a cancer cell, since EAT cells that fail to "take" when injected subcutaneously result in death when given intraperitoneally. Further, the overall well-being of the immune mechanism is an important deciding factor in the body's ability to cope with a cancer cell within its boundaries. These findings in mice show that EAT cells damaged by x-rays are successfully destroyed by intact mice, but that despite massive doses of irradiation some EAT cells must still remain capable of multiplication when implanted into hosts weakened by x-rays. With their immune mechanisms paralyzed, such mice serve in effect as in vivo tissue culture media for unopposed cancer growth.

Homeostasis of Antibody Formation in the Adult Rat

D. A. Rowley and F. W. Fitch

Passive immunization of rats with homologous anti-sheep erythrocyte serum markedly inhibited the primary antibody response to various doses of sheep erythrocytes. Inhibition was "specific" and apparently produced by either "19S" or "7S" antibody to the antigen. Passive immunization inhibited splenic hyperplasia associated with the primary antibody response. Passive immunization 24 hours after active immunization effectively inhibited the primary antibody response.

The markedly suppressive effect of specific antibody on the primary antibody response contrasted sharply with the absence of this effect on the secondary response. Antigen-antibody complexes formed in vitro elicited no measurable primary antibody response but did elicit a high secondary response. Exposure of normal spleen cells to the antibody in vivo or in vitro suppressed their response to the antigen in x-irradiated recipients. In contrast, cells from previously immunized animals transferred to x-irradiated animals produced antibody in the presence of passively given antibody. Thus, "potential antibody-forming cells" from normal animals were unresponsive to the antigen in the presence of specific antibody, while "antibody-forming cells" from previously immunized animals responded to the antigen in the presence of antibody. Presumably, antibody actively produced in small quantities by a few antibody-forming cells might inhibit antibody formation by potential antibody-forming cells. Confirmation of this suggestion was obtained by showing that some animals initially injected with small doses of antigen failed to produce measurable antibody to subsequent injections of larger doses of the antigen. Low doses of antigen capable of inducing unresponsiveness produced no measurable circulating antibody, but these doses did produce increased numbers of plaque-forming (antibody-releasing) cells in spleens of rats. Thus, the formation of specific antibody may provide a homeostatic or "feed-back" mechanism which

controls or limits production of specific antibody to the portion of the antibody-forming system previously stimulated by the antigen. This mechanism may account in part for immunological unresponsiveness produced in certain other related experimental systems.

THE MECHANISM OF TOLERANCE PRODUCED IN RATS TO SHEEP ERYTHROCYTES. I. Plaque-Forming Cell and Antibody Response to Single and Multiple Injections of Antigen

D. A. Rowley and F. W. Fitch

Previous studies suggested that an active immune response was partially responsible for maintaining immunological unresponsiveness to sheep erythrocytes. Measurement of the plaque-forming (antibody-releasing) cell response proved to be a sensitive indicator of an immune response to sheep erythrocytes in the absence of detectable circulating antibody to the antigen. The present studies were undertaken to determine whether an active immune process, measured by the plaque-forming cell response, was partially responsible for induction and maintenance of tolerance. Rats injected intraperitoneally with large doses of sheep erythrocytes beginning at the day of birth develop tolerance to the antigen. In this paper, the plaque-forming cell and antibody response to sheep erythrocytes was characterized for rats receiving a single antigen injection at various ages, and for rats which

received repeated antigen injections as adults. The dose of antigen was the same as that used to produce tolerance; the injection schedule for repeated immunizations was also the same as that used to produce tolerance.

Rats receiving a single antigen injection on the day of birth or at age 7 days had no measurable response to the antigen. Rats receiving a single antigen injection at age 17 days and sacrificed 4 days later had an unequivocal response to the antigen. The spleens had about one-tenth as many plaque-forming cells as spleens of adult animals immunized similarly, but the antibody titers were as high as titers for adult animals. Presumably the high titers of these young animals resulted from the high ratio of plaque-forming cells to body weight and blood volume. Adult animals receiving a single antigen injection had a peak or near peak plaque-forming cell response 4 days after immunization; at this time, sera contained high titers of 19S antibody and the numbers of plaque-forming cells in spleens correlated reasonably well with circulating antibody titers. 7S antibody appeared in serum 5 or 6 days after immunization. The numbers of plaque-forming cells declined progressively 2 and 3 weeks after immunization.

Repeated twice weekly, injections of the antigen in adult rats produced a marked decline and then stabilization of numbers of plaque-forming cells in spleens. Although the numbers of

plaque-forming cells were fewer, titers of 19S and 7S antibody stabilized at high levels. A progressive recovery of the plaque-forming cell response and a rise in antibody titer occurred when the interval between the last 2 injections was increased from 3 to 10, 17, or 32 days. These findings suggested that repeated closely spaced antigen injections interfered with either cell division or maturation of antibody-forming cells. As the interval between injections was increased, additional antibody-forming cells matured or were formed through cell division. Thus, relatively constant antigenic stimulation provided a mechanism for controlling or limiting the response of antibody-forming cells.

THE MECHANISM OF TOLERANCE PRODUCED IN RATS TO SHEEP ERYTHROCYTES. II. The Plaque-Forming Cell and Antibody Response to Multiple Injections of Antigen Begun at Birth

D. A. Rowley and F. W. Fitch

An active immune response to sheep erythrocytes was demonstrated in rats made "tolerant" to sheep erythrocytes by twice-weekly antigen injections beginning on the day of birth. Groups of tolerant rats were sacrificed 4 days after they had received 5 to 42 antigen injections; spleens were sampled for plaque-forming (antibody-releasing) cells and sera were titrated for antibody to sheep erythrocytes using a sensitive "plate hemolysin" technique.

During the third week of life and after the fifth antigen injection, the tolerant rats had an immune response equivalent to that of rats of similar age which had received a single antigen injection, but spleens contained only about one-tenth as many plaque-forming cells as adult animals receiving similar antigen injections. Continued antigen injections produced a marked decline and stabilization of this relatively small population of antibody-forming cells; however, the number of plaque-forming cells in the tolerant rats remained considerably elevated above the numbers of plaque-forming cells present in the spleens of non-immunized animals. The sera from all but one tolerant rat had demonstrable antibody to sheep erythrocytes in low titer. A progressive recovery of the plaque-forming cell response and rise in antibody titers occurred in adult tolerant rats when the interval between the last 2 antigen injections was increased from 3 days to 14 or 28 days.

The decline and stabilization of numbers of plaque-forming cells occurring with continued injections after the third week of life paralleled a similar decline and stabilization in rats receiving similar antigen injections as adults. Also, the recovery of the plaque-forming cell and antibody response of tolerant animals paralleled the recovery observed when the interval between injections was increased in rats receiving similar antigen injections as adults. These findings suggested that the same

mechanism controlled numbers of antibody-forming cells in tolerant and normally responsive adult animals. Repeated closely spaced antigen injections presumably interfered with either cell division or maturation of antibody-forming cells. As the interval between injections was increased, additional antibody-forming cells matured or were formed through cell division. Relatively constant antigenic stimulation provided a mechanism for controlling or limiting the response of antibody-forming cells.

The mechanism controlling or limiting the response of antibody-forming cells would not account for the stabilization of numbers of antibody-forming cells at high levels for normal animals and at low levels for the tolerant animals. Passive immunization of growing rats with homologous anti-sheep erythrocyte serum markedly inhibited the plaque-forming cell response of growing rats. It was suggested that antibody produced by the small population of antibody-forming cells in the tolerant rats provided a feedback or homeostatic mechanism which inhibited transformation of potential antibody-forming cells to antibody-forming cells. Thus, tolerance to sheep erythrocytes was induced and maintained by 2 mechanisms. One mechanism, dependent on relatively constant antigenic stimulation, limited or controlled the numbers of antibody-forming cells. The other, dependent on the production of small quantities of antibody by a few antibody-forming cells, limited or controlled the transformation of potential antibody-forming cells to antibody-forming cells.

ANTIGEN METABOLISM IN THE RAT.

I. Bovine Gamma Globulin

C. W. Pierce and F. W. Fitch

Immunization of rats with a mixture of bovine gamma globulin (BGG) and S. typhosa endotoxin resulted in a transient circulating "19S" antibody response maximum 4 days after antigen injection, as well as a slight but sustained "7S" antibody response first noted 6 days after immunization. A second antigen injection produced a brisk, mainly "7S" antibody response. Rats receiving only BGG had no measurable antibody titer after the first antigen injection; reinjection of BGG produced a secondary response similar to that of rats receiving BGG and endotoxin.

Metabolism of ^{125}I-labeled BGG was studied in rats receiving either 10 mgm BGG and 100 micrograms endotoxin and in rats given only 10 mgm BGG. Disappearance of ^{125}I-labeled BGG from the blood stream was similar for both groups of rats and followed the typical two-phase pattern described for homologous proteins. This was true despite a peak 19S antibody level on day 4 in rats receiving BGG and endotoxin. All antibody determinations were performed using the tanned erythrocyte-passive hemagglutination procedure; precipitating antibody has not been detected using agar gel diffusion. Much of the ^{125}I-labeled BGG was rather rapidly metabolized and the ^{125}I was excreted in the urine in non-protein

bound forms. The urinary excretion rates were similar in both groups of animals, about 55 per cent of the injected dose of ^{125}I being excreted by the fourth day after antigen injection.

Organ localization was studied after injection of 10 milligrams of ^{125}I-labeled BGG. At all intervals after antigen injection, the organs contained less radioactivity per milligram wet weight than did blood. The liver retained the greatest amount of activity per total organ with amounts ranging from 0.65 per cent of injected dose on day 2 to 0.085 per cent on day 12 after antigen injection. However, spleen, lymph node, and thymus had more radioactivity per gram wet weight tissue than did liver. The values for these organs ranged from 0.11 per cent to 0.17 per cent injected dose per gram on the second day after antigen injection to 0.017 per cent to 0.024 per cent of the injected dose per gram by the twelfth day after antigen injection. In general, the level of radioactivity within the organs paralleled the level of radioactivity within the blood in spite of the fact that the rats were perfused with large volumes of saline.

In autoradiographs, radioactivity was located in lymphoid follicles within the spleen. In those animals which produced antibody, radioactivity was located around developing secondary follicles. In animals not producing antibody, no secondary follicles were noted and the radioactivity was in a much less organized pattern.

We are presently studying the distribution and metabolism of one microgram of ^{125}I-labeled BGG: This is the lowest dose of BGG which, given with endotoxin, results in antibody response.

The Effect of Neonatal Thymectomy on the Immune Response of the Rat

J. C. Pinnas and F. W. Fitch

Sham-thymectomized, thymectomized, and non-operated CFN rats were immunized initially at 4 weeks of age. Antigens included sheep erythrocytes, bovine serum albumin in complete Freund's adjuvant, Salmonella typhosa flagella and flagellin, and Hemophilus pertussis vaccine. Sheep erythrocytes and flagella are particulate antigens whereas albumin and flagellin are soluble antigens. Hemophilus pertussis vaccine produces severe active cutaneous delayed hypersensitivity in the rat.

After subcutaneous immunization with bovine serum albumin in complete Freund's adjuvant, thymectomized rats had a slower rise in antibody titer than did normal or sham-operated rats. Thymectomy suppressed both the 2-mercaptoethanol sensitive antibody (19S) that is formed usually during the primary response, and the 2-mercaptoethanol resistant (7S) antibody that is formed usually during the secondary response. However, the antibody response of these same thymectomized rats to primary and secondary intravenous immunization with sheep erythrocytes was identical to

that of sham-operated and normal rats; both 19S and 7S hemagglutinin titers and plaque-forming cell response in the spleen were within the same range for all animals.

Similar results were obtained when sheep erythrocytes were used in the initial immunizations, and bovine serum albumin in the second series of antigen injections. These data indicate that neonatal thymectomy in the rat does not depress uniformly the circulating antibody response.

In current experiments, both the particulate and soluble forms of S. typhosa flagella are being used as immunizing antigen to determine if the physical state of the antigen influences the immune response of the thymectomized rat. Other thymectomized rats are being immunized with H. pertussis vaccine to determine if thymectomy influences both circulating antibody and delayed hypersensitivity to the antigenic material.

Homeostasis of Antibody Formation. The Effects of Passive and Minimal Adaptive Immunity on Homograft Survival

M. Axelrad and F. W. Fitch

The "enhancement phenomenon" observed in transplanted tumors after administration of antiserum resembles in several respects the suppressive effect of passively administered antibody on the primary immune response.

Sustained suppression of the humoral antibody response of the rat to sheep erythrocytes was observed if antigen injections were repeated at weekly intervals after a single injection of homologous anti-sheep erythrocyte serum. Sustained unresponsiveness to sheep erythrocytes was also produced in rats by immunization with very small doses of antigen which produced no measurable serum antibody titer but which did result in increased number of "plaque-forming" cells in the spleen. This specific immunological unresponsiveness was produced in adult animals using non-toxic, "physiological" methods which did not interfere with general defense mechanisms, or suppress antibody response to other antigens.

The present work is an investigation of the applicability of these procedures to the homograft reaction.

Using skin homografts in mice, we are attempting to establish tolerance across the weak sex-linked histocompatibility barrier of the C57Bl strain, and the strong H_2 barrier involved in grafting from AKR to C57Bl mice. The effect of transferring varying doses of graft-immune C57Bl female spleen cells, or multiple injections of graft-immune C57Bl female serum on graft survival in C57Bl female hosts is being studied.

Antibody Response in the Rat. Immunoglobulin Types Produced after Immunization with Sheep Erythrocytes

F. W. Fitch

Antibody appearing in the serum a few days after immunization with large doses of sheep erythrocytes is inactivated by treatment with 2-mercaptoethanol (2-ME), while a major portion of the antibody appearing several weeks after immunization is not inactivated by 2-ME. The "early" antibody migrates electrophoretically as a γ_1 globulin and is eluted as several protein fractions from DEAE-cellulose by a gradient of high ionic strength buffer. Data obtained from density-gradient ultracentrifugation suggest that both low and high molecular weight antibodies sensitive to 2-ME are present. The "late" antibody contains, in addition, a fraction with the electrophoretic mobility of a γ_2 globulin, and a low molecular weight as determined by density gradient ultracentrifugation. This antibody is eluted from DEAE-cellulose by low ionic strength buffer. Immunoelectrophoresis, however, reveals that each of the isolated protein fractions containing antibody activity consists of several distinct proteins. Present attempts to characterize the molecular types of antibody found at various times after immunization with sheep erythrocytes include the use of various gradients and buffers for elution of fractions from DEAE-cellulose; gel filtration; ultracentrifugation;

preparative electrophoresis; and radioimmunoelectrophoresis of specific ^{131}I-labeled antibody eluted from sheep erythrocytes.

The Histology of Antibody Formation

F. W. Fitch and C. R. Ahroon

Groups of rats were given intravenous injections of either 10 per cent or 0.25 per cent sheep erythrocytes (SRBC) 4 weeks apart. Agglutinin levels were determined both before and after treatment of the serum with 2-mercaptoethanol (2-ME). Spleens from these animals were fixed in Carnoy's fluid, sectioned, and the sections were stained with methyl green-pyronin. In both groups of rats, agglutinins inactivated by 2-ME reached a peak 4 days after immunization and rapidly declined; only small amounts of this agglutinin were formed after secondary antigenic stimulation. High titers of an agglutinin not inactivated by 2-ME were found from 8 to 28 days after the first immunization in the group injected with 10 per cent SRBC; this titer rose only slightly after the second immunization. Rats injected with 0.25 per cent SRBC formed only small amounts of this antibody after the first immunization, but produced relatively large amounts after the second immunization.

Sections of the spleen showed increased numbers of pyroninophilic cells in the periarteriolar lymphoid sheath beginning 1 to 2 days after antigen injection. The number of these cells

increased and then declined over the next few days. After the maximal development of cells in the periarteriolar sheath, islands of pyroninophilic cells developed in the splenic red pulp, primarily along arterioles and trabeculae.

The number of pyroninophilic cells in the splenic red pulp decreased rapidly after the sixth post-immunization day. These changes were more marked in animals receiving the larger antigen dose but were qualitatively similar in both groups. Changes in nodular lymphoid tissue were difficult to evaluate because of considerable variation in appearance in both normal and immunized rats.

The number of plaque-forming cells in the spleen correlated well with "19S" antibody levels in the circulation. There was general correlation between the plaque-forming cell response and the histologic changes. It appeared, however, that only a portion of the proliferating and differentiating cells in the spleen were able to release antibody actively at any one time; at the time of maximal histological response, only about 200 plaque-forming cells were present in a typical section. Appreciable numbers of plaque-forming cells were present in the spleens of rats several weeks after immunization with the larger antigen dose.

Current studies are attempting to determine the detailed cellular dynamics associated with the immune response.

Studies on the Inhibition of Complement by Polyinosinic Acid

S. Yachnin and D. Rosenblum

Polyinosinic acid (poly I) is a potent inhibitor of hemolytic complement (C') activity. Study of the effect of poly I on the formation and lysis of persensitized cells (EAC'....) has shown that poly I inhibits the first component of C' (C'1) without inhibiting C'1 esterase activity. The inhibition of purified C'1 by poly I has been studied using isolated C'1 subcomponents. Whole C'1 can be effectively inhibited by 10^{-3} μmol P poly I/ml and lysis of EAC'1 is prevented by preexposure to 8×10^{-4} μmol P poly I. Poly I has been shown to selectively inhibit the C'1q subcomponent of C'1 (11S component). Inhibition of C'1q and to a lesser extent of whole C'1 are reversible by subsequent addition of polyadenylic acid (poly A) which binds poly I in a hydrogen bonded helix. The effect of poly I on the total C' system in the fluid phase has been further studied using poly A as a "stopping" agent. Such studies reveal that in addition to (reversible) inhibition of hemolytic C'1 activity, poly I leads to inactivation of C'4. Inactivation of C'4 occurs only in the presence of Ca^{++} and depends upon the presence of C'1. Poly I appears to act in a manner analogous to antigen-antibody aggregates and aggregated γ-globulin, but as yet no evidence for inactivation of C'2 or C'3 by poly I has been obtained. In vivo

studies have shown that poly I is capable of "decomplementing" Wistar rats and of evoking vascular permeability in guinea pigs when injected intracutaneously.

pH Optima in Immune Hemolysis: A Comparison Between Guinea Pig and Human Complement

S. Yachnin

Certain in vitro hemolytic systems involving human erythrocytes and serum function more effectively if hemolysis is allowed to proceed in serum which has been acidified to pH 6.5. Among these systems are, hemolysis by high titer cold agglutinins, hemolysis of enzyme treated human erythrocytes, hemolysis induced in human erythrocytes by polyinosinic acid, and hemolysis of red cells from patients with paroxysmal nocturnal hemoglobinuria (PNH). In order to determine whether such "pH dependency" implies that factors other than complement (C') play a role in their hemolytic mechanism, the pH optima for classical immune hemolysis using sheep red cells sensitized with rabbit amboceptor (EA) were investigated with both human and guinea serum as a source of complement (C'). Both human and guinea pig C' function best at pH 6.5, but human C' is substantially more sensitive to pH alteration (average human C' titer at $\frac{\text{pH } 6.5}{\text{pH } 7.5} = 1.6$, guinea pig C' titer at $\frac{\text{pH } 6.5}{\text{pH } 7.5} = 1.05$). Study of the effects of pH alteration upon the formation and lysis of EA-C' component intermediates

(EAC'_1, $EAC'_{1,4}$, $EAC'_{1,4,2}$) has revealed that the enhanced potency of human C' at pH 6.5 results from stimulation of the third C' component (C'3). Thus in certain of the human hemolytic systems cited, enhanced hemolysis induced by pH reduction is a C' mediated phenomenon rather than one produced by increasing hemolysin attachment. Similarly, these observations support the argument that the C' system plays the pre-eminent role in in vitro PNH red cell lysis. That C'3 is predominantly affected by pH reduction is of interest in view of recent evidence suggesting that PNH hemolysis is a threshold phenomenon which depends upon low grade activation of fluid phase late-acting C' components in human serum.

The Initiation and Enhancement of Human Red Cell Lysis by an Activator of the First Component of Complement

S. Yachnin

Polyinosinic acid (poly I) added to a suspension of red cells (E) from patients with paroxysmal nocturnal hemoglobinuria (PNH) in normal human serum results in pronounced stimulation of hemolysis. Poly I will also initiate substantial hemolysis of normal human E in serum. The pH optimum for poly I induced hemolysis is 6.5. Exposure of E to poly I, followed by thorough washing, does not result in increased hemolysis when the cells are subsequently added to serum. Pre-exposure of serum to poly I for increasing periods of time results in progressive decrease in hemolysis when E are subsequently added; serum pre-exposed to

poly I ultimately loses the ability to support any hemolysis of PNH E. Poly I induced hemolysis is rapidly lost upon slight dilution of serum, but is not affected by prolonged in vitro storage of E. Poly I induced hemolysis is abolished by addition to serum of Na_3HEDTA. Removal from serum of any one of the four major components of complement abolishes its capacity to support Poly I induced hemolysis; removal of properdin from serum only partially reduces its capacity to support poly I induced hemolysis. The known ability of poly I to function as an activator of the first component of complement (C'1) suggests that poly I induced hemolysis results from evolution in the fluid phase of hemolytically active, late-acting labile complement components capable of injuring red cell membrane despite the lack of an antibody coat. This conclusion is supported by the observation that purified activated human C'1 or C'1 esterase also act to stimulate PNH E hemolysis in a manner similar to that of poly I. The demonstration that "indifferent" complement activation can result in red cell damage may be pertinent to concepts regarding the pathogenesis of certain acquired hemolytic anemias in man.

The Hemolysis of Red Cells from Patients with Paroxysmal Nocturnal Hemoglobinuria by Isolated Subcomponents of the Third Complement Component

S. Yachnin

Highly purified preparations of β_{1c} globulin are capable of attaching directly to red cells from patients with paroxysmal nocturnal hemoglobinuria (PNHE). The intermediate complex thus formed ($PNHE_{\beta 1c}$) in contradistinction to PNHE, is susceptible to hemolysis by high dilutions of human serum in the absence of Ca^{++} or Mg^{++}. $PNHE_{\beta 1c}$ will hemolyze in serum lacking properdin, or the first, second or fourth components of complement, but not in serum devoid of the third component of complement (C'3). Hemolysis of $PNHE_{\beta 1c}$ can be effected by purified subcomponents of C'3 (C'3b, C'3c) and the behavior of $PNHE_{\beta 1c}$ in all respects resembles that of their counterpart in classical immune lysis, $EAC'_{1,4,2,\beta 1c}$. Normal human red cells are also susceptible to hemolysis by purified subcomponents of C'3, but to a much lesser extent than PNHE. These findings confirm earlier speculation that in ordinary acid hemolysis in whole human serum, early fluid phase events in C' activation lead to direct attack of PNHE by C'3, without the mediation of cell bound complement components. The difference between normal human red cells and PNHE would appear to involve the number or accessibility of membrane sites concerned with the attachment of β_{1c} globulin, which have long been recognized as participating in the phenomenon of immune adherence.

STUDIES IN MOLECULAR BIOLOGY

STUDIES OF ERYTHROPOIETIN-INDUCED DIFFERENTIATION:

I. The Effects of Inhibitors on Hemoglobin Synthesis

O. Gallien-Lartigue and E. Goldwasser

The biosynthesis of hemoglobin in primary cultures of rat marrow cells is markedly stimulated by the presence of small amounts of the hormone, erythropoietin, in the culture medium. This effect is counteracted by actinomycin D if the inhibitor is added before the hormone or shortly after. If the cells have been in contact with the hormone for 24 hours before the inhibitor is added, the subsequently stimulated hemoglobin synthesis is resistant to actinomycin, indicating a relatively long lifetime for the hemoglobin messenger RNA. Stimulated hemoglobin synthesis is largely, but not completely, inhibited by colchicine. These observations indicate that the hormone acts via DNA dependent RNA synthesis and that the initiation of gene transcription may be among the early steps of erythropoietin action. The system marrow cells in culture:erythropoietin, is a model for the study of biochemical mechanisms underlying the process of differentiation.

STUDIES OF ERYTHROPOIETIN-INDUCED DIFFERENTIATION:

II. The Effect on RNA Synthesis

S. B. Krantz and E. Goldwasser

Erythropoietin effects an early (15 minutes) increase of RNA synthesis in marrow cell cultures. The properties of this

RNA, viz: high specific activity, sedimentation coefficient between 12 and 24S, inhibition by actinomycin D, and very rapid labeling, are those expected of messenger RNA. The hormone does not affect DNA synthesis in marrow cells for periods up to 9 hours, nor does it affect RNA synthesis by non-target cells (undifferentiated Murphy-Sturm tumor). These data fit the hypothesis that this developmental hormone acts on the transcription of previously repressed genetic loci leading to differentiation, and therefore to new biochemical functions arising in the cell.

STUDIES OF ERYTHROPOIETIN-INDUCED DIFFERENTIATION: III. Some Aspects of Induced Hemoglobin Synthesis

S. B. Krantz and E. Goldwasser

Recent work in this laboratory has shown that a simple model involving the interaction of a definite number of erythropoietin molecules with susceptible cells of the marrow resulting in increased hemoglobin synthesis does not hold. The relationship between rate of hemoglobin synthesis and number of cells in the culture is sigmoid rather than linear, as was predicted by the simple model. We have further demonstrated that only a minute fraction of the hormone present in the medium is taken up by the cells during incubation times of up to 15 hours. These two sets of data suggest that there is a cooperative effect between the potentially hemoglobin synthesizing cells. Even in the presence of erythropoietin, the rate of hemoglobin synthesis falls off to a low value starting at about 30 to 35 hours after

initiation of the culture. Since there is very little depletion of the hormone, this decline may be due to the exhaustion of some metabolite in the medium. When the medium is replaced every 24 hours, hemoglobin synthesis is maintained for an additional 24 hours beyond controls in which erythropoietin is present but the medium has not been changed. Efforts to maintain long-term hemoglobin synthesis in culture are still under investigation.

STUDIES OF ERYTHROPOIETIN-INDUCED DIFFERENTIATION:

IV. The Stimulation of Stroma Synthesis

P. P. Dukes, S. Shin and E. Goldwasser

Erythropoietin in marrow cell cultures causes increased incorporation of glucosamine-1-^{14}C into the carbohydrate moiety of an insoluble fraction consisting largely of red cell stroma. This process can be studied very simply by measurement of the radioactivity incorporated into whole cells collected on membrane filters. After a lag period of about 3 hours, the hormone induces a linear increase in amount of glucosamine incorporated for 48 hours. This stimulation is completely inhibited by actinomycin D and by puromycin, but is not at all affected by colchicine, suggesting that the hormone effect depends upon RNA and protein synthesis, but not upon cell division. Kinetic data obtained with this system suggest that the hormone acts on a small fraction of the potentially sensitive cells among those in the

marrow population. The increased incorporation with time seems to reflect the rate of production of erythropoietin-sensitive cells from those cells in other stages of their mitotic cycle.

STUDIES OF ERYTHROPOIETIN-INDUCED DIFFERENTIATION:

V. The Partial Purification of Erythropoietin

E. Goldwasser and C. Kung

In order to study the relationship between the chemical properties and biological effects of erythropoietin, it is obviously important to have available a supply of the pure hormone. For the past 8 years we have been attempting to purify this hormone from plasma derived from hemolyzed animals. Starting from plasma with a potency of .007 units per mg of protein, and using a combination of ion-exchange methods, gel adsorption, and gel filtration, we have succeeded in getting minute amounts of a fraction with a potency of about 2000 units per mg of protein -- a purification factor of about 280,000. Amounts of this preparation are too small for tests of homogeneity but there is evidence that even higher potencies can be achieved. Since supplies of anemic plasma are severely limited, the prospect of getting enough "pure" erythropoietin for the study of its chemical properties from this source, is quite dim. Another possible source is human urine from severely anemic patients. This source, while potentially large, has another problem associated with it;

the hormone found in urine is appreciably less stable than is the preparation from plasma, and frequently all activity is lost upon chromatography. This problem is still under investigation.

An extension of the Yphantis-Waugh ultracentrifuge separation-cell technique for the determination of the sedimentation coefficient and molecular weight of erythropoietin by use of the biological assay methods has been made with the collaboration of the Biological Science Computation Center. Dr. H. Landau has programmed the Lamm transport equation for solution by the digital computer with our own cell parameters. This method for molecular weight determination is now being tested with a series of proteins of known molecular weight, and we hope to extend the method to erythropoietin in the near future.

Protein Synthesis in Heart Muscle

M. Rabinowitz, R. Zak, K. G. Nair and L. DeSalle

In order to elucidate the mechanism of assembly of structural proteins such as actomyosin and mitochondrial lipoprotein, the protein synthesis systems of heart muscle from chick embryos and rats are being isolated and characterized. Ribosomal and mitochondrial systems which actively incorporate labeled amino-acids into protein have been isolated. The composition and enzymic properties of the ribosomal system are similar to ribosomal preparations from other tissues and organisms. It contains over

50 per cent RNA, and requires magnesium and potassium or ammonium ions, GTP, sRNA, and transfer enzymes for optimal ^{14}C-amino acid incorporation into protein. When maximally stimulated by polyuridylic acid, its activity is identical with that of liver ribosomes. Density gradient centrifugation reveals a 70 to 80s peak and also heavier material. It is of significance that the action of proteolytic enzymes such as trypsin and chymotrypsin leads to the dispersion of the ribonucleoprotein aggregates, as does the action of ribonuclease. The effects of ribonuclease and proteolytic enzymes are additive. This suggests that muscle ribonucleoprotein aggregates are held together by both messenger RNA and by protein, possibly by nascent polypeptide.

The mitochondrial protein synthesis system in muscle is also under study. It differs from the ribosomal system in that it is not inhibited by ribonuclease, but is inhibited by actinomycin and chloramphenicol. Characterization of mitochondrial DNA and RNA and evaluation of their role in mitochondrial protein synthesis is now in progress. DNA has been isolated from mitochondria purified by isopyknic density gradient centrifugation. Its base composition is being compared to that of nuclear DNA by measuring their buoyant densities in the analytical ultracentrifuge. ^{3}H-thymidine incorporation into mitochondrial DNA has been measured. The presence of DNA and RNA polymerase systems in mitochondria is being investigated, and the further characterization of the mitochondrial protein synthetic apparatus is under study.

Studies on RNA Biosynthesis

S. B. Weiss

We are continuing our efforts to understand the enzymatic mechanism by which purified microbial RNA polymerase catalyzes the synthesis of RNA molecules. This enzyme is interesting because it utilizes DNA as a template for ribonucleotide polymerization and also because it appears to be responsible for most of the cytoplasmic RNA found in living organisms.

In vivo experiments have shown that only one of the two DNA strands is transcribed by cellular RNA polymerase, however, our purified enzyme has been shown to transcribe both DNA strands in vitro. We now know that the integrity of the DNA template is important for single strand transcription, but we also believe that enzyme configuration (its secondary and tertiary structure) is also important. To gain further insight into this problem, we are attempting to modify our enzyme purification procedure so that the polymerase preparation will transcribe only one DNA strand in vitro. We then hope to study its physico-chemical properties (sedimentation characteristics, electron microscopic analysis, etc.) and compare the physical properties of this enzyme to other enzyme preparations which transcribe both DNA strands. Our analysis suggests that RNA polymerase is very large with a molecular weight in the range of 500,000. Proteins of this size must

consist of subunits. It is possible that alteration of these subunits could endow this enzyme with properties different from those normally associated with its native condition.

We are also attempting to identify specific cytoplasmic RNA molecules; mainly the so-called 45S and 5S RNAs. It is believed that the 45S RNA found in mammalian extracts is a precursor of ribosomal RNA (26S and 18S) and that the 5S RNA may be a precursor of soluble RNA (4S RNA). By utilizing RNA-RNA hybridization techniques worked out in this laboratory, we may be able to determine whether these RNA components are indeed precursors of specific, well identified cytoplasmic RNA molecules.

Studies with Polycyclic Aromatic Hydrocarbons

S. B. Weiss, W.-T. Hsu and J. W. Moohr

That RNAs and DNAs prepared from numerous RNA-containing and DNA-containing viruses can be infectious has been established by a number of investigators. Various polycyclic aromatic hydrocarbons are known to be carcinogenic, although attempts to show that they have any biological effect on lower forms have been mostly unsuccessful. Recently, we observed that certain hydrocarbons, when included in systems containing infectious nucleic acid and bacterial protoplasts, inhibit the production of bacteriophage. Under appropriate conditions, the inhibition is 90 per cent or greater and the hydrocarbon concentration required to obtain this level of inhibition is in the order of

10^{-4} - 10^{-5} M. An interesting aspect of this study is the observation that only certain hydrocarbons exert this virus inhibition, primarily those which are known carcinogens. The correlation between carcinogenic activity in higher animals and virus inhibition in bacterial protoplasts is rather marked. Although we cannot say that the hydrocarbon action is the same for both phenomena, we think that this simple system will lend itself towards elucidating the mechanism by which viral growth is impaired. Preliminary results show that the "active" hydrocarbons do not impair the host cells (bacterial protoplasts) from synthesizing DNA, RNA or protein even though virus replication is inhibited. It may be that viral nucleic acid and viral protein can be made in the presence of hydrocarbon but that somewhere along the pathway for transmission of genetic information a transcription or translation error in the synthesis of viral nucleic acid and/or viral protein has been made. This error may be lethal since it could result in abortive phage production. Our current studies are concerned with the mechanism by which hydrocarbons act in this system. The possibility that these agents may act as mutagens would be novel and is currently under study.

EXPERIMENTAL AND CLINICAL STUDIES OF CELL DIFFERENTIATION

Physiological Studies of Primitive Hemopoietic Cells

C. W. Gurney, D. Hofstra and A. Mangalik

The action of erythropoietin on primitive hemopoietic cells is to induce differentiation into the red cell series. This process can be measured quantitatively, and therefore may be employed in an inquiry into the nature and proliferative potential of these cells. Three separate experiments are presented which suggest the multipotential nature of the primitive cell upon which erythropoietin acts. First, when erythropoiesis has been eliminated by transfusion, the pool of primitive cells which is potentially responsive to erythropoietin is not dormant, but rather is a dynamic pool, as demonstrated by the incorporation of tritiated thymidine into proerythroblasts induced by erythropoietin, even when the tritiated thymidine is administered before the erythropoietin. Second, the pool of erythropoietin-responsive cells in the sub-lethally irradiated plethoric mouse recovers in the absence of any red cell production. Third, the recovery of the pool of erythropoietin-responsive cells is retarded when irradiated animals are subjected to an inflammatory process characterized by a massive polymorphonuclear infiltration (and presumably, increased leukocyte production).

The pattern of proliferation of a small group of protected erythropoietin-responsive cells was followed by shielding the

spleens of plethoric mice subsequently given large doses of irradiation, and observing the response to a challenging dose of erythropoietin administered to different groups of animals at different times. Proliferation of these cells was rapid, with an overshoot to 140 per cent of normal on the eighth day. Organ uptake of radioiron indicated that the proliferation took place initially in the spleen, but in a few days after irradiation the erythropoietin sensitive cells had migrated out to the bone marrow.

These results enable us to present formulations as to the regulatory processes governing the size of the pool of cells responsive to erythropoietin, and the techniques utilized in these studies, in conjunction with methods employed by other participants of this conference, should ultimately lead to an elucidation of these regulatory processes.

Relationship Between Duration and Intensity of Hypoxia and Erythropoietic Response

C. W. Gurney

Mice are rendered plethoric after 3 weeks of hypoxia, hematocrits exceeding 70 per cent. After 5 days at ambient conditions, erythropoiesis as determined by morphology, reticulocyte count, or ^{59}Fe uptake, is virtually eliminated. Assuming that erythropoiesis is controlled by erythropoietin, we can

determine the duration or intensity of hypoxia necessary for erythropoietin elaboration by measuring erythropoietic response in such mice after brief returns to a hypoxic environment. This response is quantitated by the 78-hour incorporation of an intravenous tracer dose of ^{59}Fe. One hour at 0.5 atmosphere produces progressively increasing responses in all animals. If duration of hypoxia is constant, the response increases rapidly as pressure decreases below 0.5 atmospheres. A small erythropoietic response can be observed after exposures of only 15 minutes to the maximal hypoxia compatible with life in these animals (0.25 atmospheres). The study demonstrates the precision with which an erythropoietic response may be equated with the duration and intensity of hypoxia. It also indicates the sensitivity of the blood-forming system to hypoxia, the stimulus which acts through erythropoietin production to regulate the rate of erythropoiesis. This mechanism is sufficient to account for continuous blood production in normal ("functionally anemic") animals and man.

Quantitation of Erythroid Hypoplasia in Mice Following Irradiation

C. W. Gurney, D. Hofstra, E. Simmons and C. Newton

The erythropoietin tolerance test has given reliable data in the plethoric mouse. It has been employed in the present study to compare radiation damage of the primitive erythropoietin-

sensitive cell in 10 strains of mice. The results have been compared with radiation sensitivity as determined by LD_{50} in the same strains.

Young adult virgin female mice were hypertransfused. After erythropoiesis had ceased, different groups were given different doses of whole body radiation, immediately followed by a standard subcutaneous challenge of 3 units of erythropoietin. Marrow responsiveness to this stimulus was determined by incorporation of a tracer dose of ^{59}Fe given 2 days after erythropoietin. Cf No. 1 mice served as a standard. In 15 experiments the response following 150 r in this strain was 28 per cent (1 S.D. = 4.1 per cent) of the response in non-irradiated controls. The radiation doses which reduced the response to 10 per cent of the non-irradiated control in 10 strains varied from 110 r in the DBA/2 mouse to 225 r in the $(C3H \times 101)F_1$ hybrid. The slopes of the dose-response curves could not be related to the LD_{50}s for the various strains studied. It is concluded that erythropoietin responsiveness following irradiation measures a different aspect of radiation damage than does LD_{50}. This may be useful in quantitating radiation damage, particularly in the dose range from 0 to 300 r.

The Erythropoietic Effect of Testosterone in the Plethoric Mouse

C. W. Gurney and W. Fried

The sex difference in red cell values, and an unpredictable response to androgen therapy in patients with refractory anemias led us to initiate quantitative studies into the effect of androgens on erythropoiesis.

A single injection of 1.0 mg of testosterone intramuscularly exerts an erythropoietic effect in the transfusion-induced plethoric mouse, as indicated by an 8-fold increase in the 72-hour incorporation of radioiron by newly formed red cells in testosterone treated animals. Larger single doses of testosterone fail to give responses greater than those obtained with 1.0 mg and a single injection of less than 0.5 mg is ineffective.

Red cell formation cannot be suppressed as completely in male mice by hypertransfusion, as it can be in the female. In such plethoric mice, the female is more sensitive to testosterone: Following two 2.5 mg injections of testosterone, ^{59}Fe incorporation by newly formed red cells increases from 1.1 per cent to 21.9 per cent in females, from 4.7 per cent to only 7.8 per cent in males. This difference in response is not a function of sex-determined characteristics of the marrow cells, since the response to testosterone is independent of the sex of the donor supplying the marrow cells used to save lethally irradiated polycythemic mice.

A synergistic effect of testosterone and small doses of erythropoietin is observed, i.e., 0.15 units of erythropoietin produces 6.3 per cent iron incorporation, two successive 2.5 mg doses of testosterone lead to 11.6 per cent iron incorporation, and the combination of erythropoietin and testosterone in these doses leads to 25.5 per cent incorporation. A similar synergistic effect of testosterone and short periods of hypoxia is also noted.

For demonstration of an erythropoietic stimulating effect, the optimal time of radioiron injection in the polycythemic mouse is 2 days after administration of erythropoietin, but 4 days after testosterone. Hence, it is concluded that testosterone does not exert a direct erythropoietin-like effect on primitive marrow cells susceptible to the stimulus for differentiation into the red cell series. Four days after a single injection of 5 mg of testosterone to normal mice, plasma shows a demonstrable titer of erythropoietin on bioassay, hence it may be concluded that one mechanism of testosterone action is via stimulation of erythropoietin production.

The demonstration and quantitation of the androgenic effect in plethoric mice provides a model in which the influence of these substances and their mechanism of action on erythropoiesis may be investigated.

An Erythropoietic Defect in a Congenitally Anemic Mouse

A. Kales, W. Fried and C. W. Gurney

Anemia occurring in mice by mutation at the w-locus has been the subject of several reports. The present abstract is concerned with an anemia occurring in the C57 Black mouse that differs in several characteristics from those previously described.

The heterozygote mutant is black with a grayish tinge on the ventral surface and a white patch on the head. It has a normal blood count.

The homozygote mutant is a slow-growing white mouse with black eyes. It has a macrocytic anemia associated with a mildly elevated reticulocyte count and a normally cellular marrow. This condition, obtaining in the presence of anemia, suggests relative insufficiency of erythropoiesis. Survival of the homozygote erythrocytes in Black hosts, and of Black erythrocytes in homozygote hosts is normal.

The anemic (homozygote mutant) mouse loses 3 per cent of its blood volume from the gastrointestinal tract daily, although a gross lesion has not been found. This blood loss is not due to iron deficiency, since the system does not respond to iron therapy. Following transfusion, the mutant mouse is less responsive to hypoxia than is the Black mouse, and responds to injections of erythropoietin only when these are given in large doses. Endogenous erythropoietin titers are extremely high in the mutant and

are elevated proportionally higher by a hypoxic stress than are the titers in the parent (heterozygote) strain. This condition suggests that the stem cells are defective in the ability to respond to erythropoietin. We must emphasize here that the data do not suggest that erythropoiesis is regulated otherwise than by erythropoietin, since administration of the hormone in high titer is effective. The observation that marrow cells from mutant mice are poor colony formers when transfused into x-irradiated Blacks provides additional support for the existence of a stem cell defect.

In summary, we are describing a mutation that manifests itself by occult gastrointestinal bleeding and defects of the hematopoietic stem cell.

Autoradiographic Studies of Human Chromosomes

J. Rowley

Labeling of chromosomes with ^{3}H thymidine during the DNA synthetic period has yielded significant information regarding the sequence of chromosome replication in animals and man. In studies of autoradiographs of thymidine-labeled normal human female chromosomes, it has become apparent that one of the two X chromosomes replicates its DNA later than its homolog and later than almost all the autosomes. This late synthesizing X chromosome is not present in the normal human male, nor is it present in

abnormal females with Turner's syndrome who have only one X chromosome, (and therefore only 45 instead of the normal 46 chromosomes). In collaboration with Drs. Lajtha, Muldal and Gilbert of Manchester, England, and Dr. Lindsten of Stockholm, Sweden, similar studies on a boy with 49 chromosomes and an XXXXY sex complement (i.e., 3 extra X chromosomes) have revealed the presence of 3 late labeling X chromosomes. Some females with Turner's syndrome have 46 chromosomes including 2 X chromosomes, one of which is structurally abnormal. We have studied three such structurally abnormal X's: 1) a presumptive isochromosome for the long arm of X; 2) a deletion of the short arm of the X; and 3) a ring X chromosome. In each case, the morphologically abnormal X is late-labeling. Correlating this finding with measurements of the DNA content of the sex chromatin mass found in the nuclei of these patients, we can say that the late synthesizing X chromosome forms the sex chromatin mass in the interphase nucleus. It is interesting to speculate on the significance of these findings in relation to the Lyon hypothesis of X-inactivation.

Results of Chromosome Analysis in Patients with Primary Refractory Anemia

J. Rowley and R. K. Blaisdell

A notable contribution to leukemia research was the description by Nowell and Hungerford of the minute (Ph^1) chromosome in metaphases of cultured blood cells of patients with chronic

myeloid leukemia. The fairly consistent occurrence of the Ph^1 chromosome in blood or bone marrow cells from patients with chronic myeloid leukemia has been confirmed by many investigators. No consistent karyotypic pattern has emerged from studies of acute and other forms of chronic leukemia, treated or untreated. However, approximately one-half of all cases of acute myelogenous leukemia have abnormalities in chromosome number or morphology.

Since cases of refractory anemia or pancytopenia may terminate with acute leukemia, study of chromosomes in such cases throughout the course of the illness should be helpful in understanding its pathogenesis. Three questions may be asked:

1) While no consistent chromosome abnormality has yet been found in acute myelogenous leukemia, is a consistent abnormality in number or morphology present in a specific clinical sub-group such as the patients with primary refractory anemia?

2) If a chromosome abnormality does occur, when does it appear in relation to the clinical course and specific features of the disease?

3) If there is a chromosome abnormality in some patients with refractory anemia, does the absence of the chromosome aberration in other patients have any clinical value in distinguishing those who are not pre-leukemic?

Results to date reveal that of the 9 patients whose bone marrow contained sufficient metaphases suitable for analysis, 4

were normal, 2 were clearly abnormal, and 3 contained cells showing inconsistent abnormalities of uncertain significance. One of the patients has died, of pneumonia; analysis of his chromosomes revealed that 10 per cent of the cells showed inconsistent abnormalities and 10 per cent were tetraploid. Autopsy failed to disclose evidence of leukemic transformation. None of the surviving patients have shown clinical signs of leukemia.

On the basis of these early findings it appears that the answer to the first question is no. The answer to questions 2 and 3 must await a longer period of study.

STUDIES ON THE BLOOD

The Kidney and Erythropoietin Production

L. O. Jacobson, E. K. Marks and E. O. Gaston

A number of investigators have demonstrated that erythropoietin is produced by the kidney of mice, rats, rabbits and dogs. Studies of patients with severe renal disease and anemia suggest that the human kidney is likewise involved in erythropoietin production.

Some observers have raised questions of relevance: Does the kidney normally function as the primary site of the erythropoietin production necessary for the maintenance of the steady state of the erythron; or are the kidneys of only secondary importance to some other site or sites in the body responsible for production of the hormone under physiological conditions. The evidence to answer either of these questions is not available.

The plasma of bilaterally nephrectomized rodents (mice, rats, rabbits) subjected to hypoxic anoxia (simulated altitude of 21,500 ft.) for 12 hours regularly contains about one-tenth the erythropoietic activity (assayed in a transfusion-induced polycythemic mouse) observed in non-nephrectomized controls similarly stressed. This reproducible observation clearly shows that bilaterally nephrectomized rodents subjected to this degree of anoxia have the capacity to produce more erythropoietin than is normally found in intact rodents in a steady state, but under

these conditions obviously maintain only a fraction of the erythropoietin production capacity observed in the intact controls.

Earlier experiments in this laboratory indicated that removal of a number of organs and tissues (including pituitary, adrenal, thyroid, stomach, spleen, etc.) did not appreciably reduce erythropoietin production in rodents with intact kidneys in response to anemic anoxia or cobaltous chloride administration.

In retrospect, these extirpation studies did not preclude the possibility that one or more of these organs or tissues might contribute minimally to erythropoietin production, and would not have been detected in the presence of an intact major producer, namely the kidney. We have accordingly begun a systematic re-examination of this problem, utilizing a variety of techniques including extirpation of organs and tissues and/or irradiation.

We have observed that the administration of 5000 r of x-irradiation to both kidneys of the mouse, using a columnated beam immediately prior to placing the animal in a simulated altitude of 21,500 ft. for 12 to 16 hours, is as effective in reducing the response to hypoxic anoxia measured in erythropoietin plasma titer as is bilateral nephrectomy. Under these conditions it is of significance that the minimal plasma erythropoietin titer observed is comparable to that of the bilaterally nephrectomized animals subjected to hypoxic anoxia.

We have also observed that the administration of 6,000 r whole-body irradiation reduces erythropoietin production in the mouse in response to hypoxic anoxia (simulated altitude 21,500 ft) to levels significantly below that observed in bilaterally nephrectomized animals subjected to this hypoxic anoxic stimulus and significantly below that observed in mice given 5000 r to the kidneys and subjected to hypoxic anoxia.

The technique of selective irradiation of parts of the body combined with extirpation should assist us in delineating the extra renal site or sites of erythropoietin production.

Effects of Long-Term High Pressure Oxygen in Animals

E. L. Simmons, J. Doull, L. O. Jacobson and E. K. Marks

Despite the body's need for oxygen to sustain life, the toxicity of pure oxygen under pressure is well established. The use of hyperbaric oxygen has assumed increasing medical importance in recent years in such diverse fields as tumor treatment, surgical procedures, the control of gas gangrene, revival of stillbirths, etc. A better understanding of oxygen metabolism is essential in space medicine, since our astronauts breathe pure oxygen under positive pressure in the capsules, and for research in improved submarine and diving procedures.

At the Argonne Cancer Research Hospital, in addition to using high altitude chambers to produce polycythemia in animals,

and as a tool to study the effect of reduced oxygen tension on blood formation, we are also subjecting animals to long-term treatment with high pressure oxygen. It has been reported that short-term exposure of mice to O_2 at 90 lbs. pressure results in hemolytic anemia, and we have observed that this can be produced by chronic exposure at 15 lbs. We have also determined that continued exposure to oxygen will saturate the blood and result in a reticulocyte-free state. This effect was achieved in some young CF No. 1 mice after exposure for 5 days to a cycle of 15 lbs. of O_2 for 5 hours followed by 1 hour in normal atmospheric air. Such treatment proved to be extremely toxic and most of the mice usually died before these blood changes occurred. It therefore became necessary to explore ways of keeping animals alive for longer periods in oxygen.

It is not considered safe to expose human patients to hyperbaric O_2 for longer than 2 hours during every 8-hour period. In our long-term animal exposures it proved possible to shorten considerably the rest period in air between successive oxygen exposures. Interestingly enough, however, 2 hours was still about the longest period for continuous exposure to hyperbaric oxygen before fatal damage occurred. Rats and mice lived many months in pure O_2 at normal atmospheric pressure, and also in a 2 hours O_2 at 15 lbs. pressure: 1 hour air cycle. However, a cycle of 3 hours O_2 at 15 lbs.: 1 hour air was quickly fatal.

Oxygen-air ratios such as 2:1/2, 2-1/2:1, 2-1/2:1/2, etc. are being run to ascertain whether survival is determined by the exposure time O_2 or the recovery time in air. To date we have been unable to "condition" mice to an increased period of oxygen by gradually lengthening exposure time. Chemical treatment with Vitamin E, mercaptoethylamine, Tris buffer, etc., is being explored for possible protective action. Tests with rabbits, rats, and mice have shown that animals with larger bodies are more sensitive to oxygen effect than are smaller-bodied species. Strain and sex differences, and effects of age versus body weight are also under study.

THE REGULATION OF IRON ABSORPTION: Hepatic Regeneration as a Stimulus to Increased Iron Absorption

G. A. Mendel

It is clear that because of ineffective excretory mechanisms, body iron content in man is largely determined by the quantity of iron absorbed from the gastrointestinal tract. When physiologic amounts of iron are involved, our studies have indicated that the quantity of iron absorbed varies directly with the rate of erythropoiesis and inversely with the hemoglobin level and body iron content, and that these factors have additive effects on the mucosal transport of iron. Unexplainable by these regulatory

factors are the increased iron absorption that occurs in hemochromatosis, in some cases of hepatic cirrhosis, and during periods of rapid growth and pregnancy. To determine whether increased proliferation of some tissue other than the erythroid marrow would influence iron absorption, and because hepatic regeneration is a prominent feature of hemochromatosis and hepatic cirrhosis, studies of iron absorption have been undertaken during the period of rapid liver regeneration that follows partial hepatectomy in the mouse.

These studies have demonstrated the following: There is a transient 2- to 3-fold increase in iron absorption following partial hepatectomy. This increase in absorption is not explainable on the basis of the anemia, increase in erythropoiesis, or reduction in body iron content inherent in the operative procedure. The increase occurs in the face of some degree of iron overload. In animals subjected to fractional hepatectomies, the increase in absorption varies with the amount of liver removed and thus with the magnitude of the regenerative response. The increase in iron absorption occurs as a wave and is detectable for a period of only 8 to 10 hours, taking place approximately 24 hours prior to the onset of the maximal wave of mitotic activity, at a time when there is an influx of the materials required for DNA synthesis and cellular division into the remaining hepatocytes.

These studies demonstrate that increased proliferation of hepatocytes can serve as a stimulus to increased iron absorption, and suggest that this phenomenon is related to the cell cycle. Further studies of the specificity of this response, in terms of other proliferating tissues and other nutriments, as well as experiments to support the thesis that the observed increase in iron absorption is related to DNA replication and cell cycle are planned or in progress.

Biochemistry of Biological Membranes: Studies on Red Blood Cells

A. Tarlov

The problem of active transport of metabolites across cellular membranes is being approached by an analytical study of membrane components, their rates of turnover, and possible changes during active transport.

The mammalian red cell, lacking a nucleus, is not capable of assembling complex molecules from their small precursors. On the other hand, it is known that the complex lipids in the red cell membrane do turn over at a significant rate. Not only do the intact complex lipids turn over but in addition, there is significant turnover of the fatty acid constituents alone. This turnover, it appears, occurs by the exchange of complex lipids or fatty acids between the red cell and the plasma lipoproteins. Much

information must be obtained before the significance of this exchange process to cell viability and human disease can be evaluated. We propose to study the kinetics of the exchange process, the cellular enzymes which effect this exchange, the source of energy required, and the factors in the plasma which are necessary to support this exchange. It is anticipated that the presence in the plasma of inhibitors of the exchange rate, or the absence of certain molecules which are necessary for the support of the exchange may be responsible for any anemias commonly seen in medicine due to such factors as bacterial or viral infections, liver disease, renal disease and others.

Recent studies suggest that the complex lipids are not distributed evenly throughout the entire red cell membrane, but that certain classes of lipids may be restricted in their location to one area, i.e., concave central area, whereas other classes may be restricted in their location to the curved equatorial areas. Perhaps membrane functions, such as active transport, immunologic properties, etc., are similarly localized. An approach to this problem will be made using tritium-labeled lipids, and tritium-labeled transportable or immunologically reactive substances with radioautography on preparations for light and electronmicroscopy.

In Vitro Studies of Human Blood Lymphocytes in Lymphoproliferative Disorders

R. K. Blaisdell

The precise nature of the abnormal proliferation which characterizes lymphocytic leukemia and lymphosarcoma, and the relationship of the kinetics and functions of the involved lymphoid cells to clinical manifestations such as tumefaction, infiltration of non-hemopoietic tissues, anemia, fever, superimposed infections, wasting, and remissions, are problems largely unresolved.

The availability of patients with these disorders for long-term observation, the ready accessibility of the blood for repeated sampling, and the application of newer methods for cell culture, have provided the opportunity to determine what relationships blood lymphocyte behavior in vitro might have to the clinical features of these illnesses.

Methods for rapid separation and in vitro culture of blood lymphocytes have been devised. Following the addition of phytohemagglutinin (PHA) to cultures of cells from normal subjects, the following have been observed: transformation of some lymphocytes into larger cells with basophilic cytoplasm resembling blasts and plasma cells; mitosis and uptake of tritiated thymidine (^{3}H-T) by small, as well as larger, lymphocytes, demonstrated by autoradiography; and a small, but significant, increase in gamma

globulin from cells, and the culture medium, determined by diethylaminoethyl-cellulose separation, electrophoresis, and carbon-14-lysine incorporation experiments.

These results suggest that under appropriate conditions, some normal circulating lymphocytes are capable of proliferation and antibody formation; that in these processes, lymphocytes reveal their relationship to plasma cells, formed elements that have previously been considered to belong to a separate cell lineage; and that in vitro cell kinetic patterns may lead to basic understanding of the nature of the lymphoproliferative disorders.

Studies to date of blood from 2 patients with active chronic lymphocytic leukemia and 2 out of 4 patients with lymphosarcoma reveal a considerably smaller proportion of cells undergoing transformation and exhibiting ^{3}H-T uptake and mitosis after PHA stimulation.

Additional experiments, using immunoelectrophoresis for determination of the types of immune globulins synthesized, and immunofluorescence to identify the cell types responsible, are in progress.

Studies of Mouse Leukemia Viruses

G. B. Humphrey and E. Goldwasser

Two mouse leukemias previously not known to be induced by viruses have been studied. The P-1534 leukemia of DBA/2 mice can

be prepared in a cell-free form by sonic breakdown of spleen cells from leukemia-carrying mice. Leukemogenic activity of this preparation seems to be associated with the large particulate fraction of the cell, and has resisted attempts at purification. The L-4946 leukemia passed to CF No. 1 mice is freed from cells by simple homogenization, and activity is found in the 100,000 g supernatant fraction. This leukemia virus has been purified about 80-fold, and appears to be an RNA containing material.

Effect of 5', 5', 5'-Trifluoroleucine on Transplanted Mouse Leukemias

E. L. Simmons, N. Larkin, C. Pierce,
H. S. Anker and O. M. Rennert

Experiments by Anker and Rennert have shown that the leucine analog 5', 5', 5'-trifluoro-D, L-leucine (TFL) can, without adaptation, replace at least half the leucine residues in the bacterial proteins of leucine auxotrophs of Escherichia coli. When mice inoculated with Ehrlich ascites tumor were treated with TFL, such animals lived a few days longer than controls. It seemed desirable, therefore, to explore the effect of TFL on a variety of transplanted mouse leukemias.

The following nationally known tumors were selected for testing: L-4946 ascitic leukemia in AKR and CF No. 1 mice, P-1534 lymphatic leukemia to DBA/2 and also to 500 r-weakened CF No. 1,

L-1210 in DBA/2 and its hybrid offspring $B6D2F_1$, and lymphosarcoma 6C3HED in C3H/Anf. In addition, several new tumor passages were started in our laboratory from AKR mice in which the onset of spontaneous leukemia was evident. These were transferred several times to young healthy AKR mice to be sure of their viability before inoculation of mice to be treated with TFL. Cell suspensions were prepared by gentle grinding of spleen lymph nodes and thymus in isotonic saline, and aliquots containing 10^2 to 10^6 cells/ml were injected intraperitoneally. The lymphosarcoma was injected intramuscularly in the hind leg after dissociation of the tumor tissue in saline.

Many variations in TFL treatment were tried, including starting time of treatment following inoculation, frequency of treatment, portal of administration, and dosages ranging between 0.5 and 2.0 mg/g of body weight.

In several of the combinations (see Table 1), TFL was found to prolong the survival of mice with a number of different leukemias. With very invasive tumors that kill rapidly, survival was less significantly prolonged, the increase in survival time of treated animals being apparently inversely proportional to the malignancy of the leukemia.

The hematologic observations in AKR and CF No. 1 inoculated with L-4946 were of interest since animals treated with TFL did not show the marked terminal anemia present in the controls: the

Table 1. Effect of TFL on Survival with Various Transplanted Mouse Leukemias

Type of leukemia	No. of mice	Strain	TFL	Mean survival (days)	P*
AKR	16	AKR	0	8.5 ± 0.2	0.3
passage	7	AKR	+	14 ± 2.0	
AKR	16	AKR	0	9 ± 0.1	
passage	7	AKR	Methotrexate	9.5 ± 0.2	0.9
	8	AKR	+	10.5 ± 1.0	0.75
	8	AKR	TFL + 200 r	12 ± 1.0	0.55
P-1534	10	DBA/2	0	9.5 ± 2	
	9	DBA/2	Methotrexate	8 ± 0.5	-0.75
	10	DBA/2	+	12.5 ± 1	0.6
6C3HED	10	C3H/Anf	0	20 ± 0.5	
	5	C3H/Anf	+	25 ± 1.0	0.7

*By difference of means on *t* test.

hematocrit value in leukemic AKR was 20, while that in the TFL-injected group was 41. Cellular examination of control blood showed anisocytosis and marked hypochromia, changes that were not present in TFL-injected mice.

In additional pilot experiments, TFL was found to have no beneficial effects on mammary carcinoma in C3H mice, or on transplanted myeloma X-5563 also in C3H mice. Blood serum from the latter mice showed a marked gamma globulin peak, the presence or

size of which was not affected by daily treatment with TFL. TFL treatment exerted no beneficial effect on normal onset and severity of spontaneous leukemia in the AKR colony.

Survival of leukemic mice was not improved when trifluorovaline was substituted for TFL. This compound is far more toxic than TFL and leukemic mice are especially sensitive to it, so that additional exploration of method of administration is needed.

GENERAL METABOLIC STUDIES

Uric Acid Metabolism in Wilson's Disease

L. B. Sorensen, R. Reilly and A. Kappas

Hypouricemia represents one of the biochemical manifestations of Wilson's disease. In this study uric acid-2-^{14}C was intravenously administered to 2 patients with this metabolic disorder in order to study pool size and turnover of this purine and cumulative urinary recovery of injected material before and after therapy with penicillamine. Pretreatment values in 1 patient were: pool, 217 mg; plasma uric acid, 0.9 mg per cent; turnover, 477 mg/day; uric acid clearance, average 32.6 ml/minute; and cumulative recovery 95.9 per cent (recovery of injected dose in normals is about 2/3). After 5 months therapy with clinical improvement values were: pool, 300 mg; plasma uric acid, 2.7 mg per cent; turnover, 426 mg/day; clearance, average 9.5 ml/minute; and cumulative recovery, 89.9 per cent. In the second patient, pretreatment values were: pool, 370 mg; plasma uric acid, 2.0 mg per cent; turnover, 720 mg/day; uric acid clearance, 24.0 ml/minute; and cumulative recovery, 86.8 per cent. After 19 months treatment with marked clinical improvement values were: pool, 486 mg; plasma uric acid, 3.0 mg per cent; turnover, 734 mg/day; clearance, average 14.1 ml/minute; and cumulative recovery 72.6 per cent. Pool size, renal clearance, cumulative

recovery of injected dose, and plasma uric acid were markedly abnormal in both patients before therapy; following treatment and subsequent clinical improvement, these chemical and physiological indices reverted towards normal. These parameters of uric acid metabolism provide useful objective measures of the beneficial effects of penicillamine therapy in Wilson's disease associated with renal-tubular dysfunction; they may also serve as important adjuncts to direct estimation of liver copper in evaluating clinical status in general in this disorder.

Molybdenum-99, A New Isotope for Scintillation Scanning of the Liver

L. B. Sorensen

Recent studies have shown that molybdenum-99 injected in a single tracer dose as ammonium molybdate disappears rapidly from the circulation of man. Six hours after injection of carrier-free material into normal subjects, the blood level of ^{99}Mo falls to less than 1/300 of the initial concentration. By use of collimated probes, as well as by postmortem radioassay of tissues, this rapid clearance has been shown to be due to a selective concentration of molybdenum in the liver.

From excretion data it is estimated that the uptake of ^{99}Mo by the normal liver is about 80 per cent when carrier-free material is injected. The biological half-life of ^{99}Mo determined from

whole-body counts is about 20 days. Studies in rats have shown that labeled molybdenum is incorporated as a non-dialyzable component of the xanthine oxidase molecule. In man, this enzyme is chiefly -- perhaps exclusively -- located in the liver.

The specificity of the liver for molybdate permits scanning of the organ for which purpose the 0.140 MeV γ radiation of the daughter technetium-99m is particularly suitable. Good visualization of the liver is obtained when scans are done 24 hours after injection of 40 μc of ^{99}Mo. At this time, maximum build-up of technetium-99m has taken place in the liver. Space-occupying lesions are readily visualized. In diffuse hepatocellular diseases, the liver accumulates less of the administered dose of ^{99}Mo, leaving more of the isotope available for urinary excretion.

Molybdenum-99 has several advantages over colloidal gold and ^{131}I-labeled rose bengal: 1) it accumulates in the hepatic parenchymal cells and its uptake portrays effectively disease states of parenchymal cells; 2) the concentration of the tracer does not change during the interval of the scan since the isotope has a long biological half-life; and 3) there is superior scanning resolution due to the softer γ-radiation of technetium-99m.

Steroid Studies

A. Kappas, F. Katz and R. H. Palmer

The major research interests of this program center on steroid metabolism and pharmacology, with particular emphasis on study of the biological properties of metabolites derived from the *in vivo* degradation of adrenal and gonadal hormones -- a line of investigation which has demonstrated that the extensive chemical transformations which steroid hormones undergo *in vivo* (including conjugation) do not necessarily "inactivate" them but may lead to the formation of new compounds having novel and potent biological activities, certain of which have relevance to clinical medicine. A new class of fever-producing agents has been described. These pyrogens are steroid metabolites of the 5β-H (A:B *cis*) class and are derived from the endogenous transformations of precursor hormones, none of which display this biological effect. Extensive studies of these 5β-H compounds (previously considered "inert") have dealt with the characteristics of the febrile reaction which they induce in man; the species specificity of this action; and its structural determinants. The mechanism by which 5β-H steroids produce fever is not yet clear; no evidence has been adduced to implicate the "leukocyte pyrogen" in the process of thermogenesis. Participation of steroid pyrogen in the mechanism of clinical fever has been demonstrated in studies by others (Bondy, Yale University) in periodic fever;

steroid pyrogen action has been related to the mechanism of fever in certain patients with the adreno-genital syndrome and liver disease. In addition, further studies on steroid fever are being conducted in leukopenic patients, and the species specificity and characteristics of the inflammatory reaction induced by these compounds are being examined in experimental animals; the hemolytic properties of these neutral 5β-H steroids have also been studied. Among structurally related derivatives of cholesterol, certain C24 steroid (bile) acids also have fever-producing and hemolytic properties. Lithocholic acid, the most potent of these breakdown products of cholesterol, has been of particular interest to us and studies are being conducted on its metabolic degradation; its cirrhosis-producing activity; and its newly discovered role in the experimental production of gallstones in animals.

Estriol, a major metabolite of natural estrogen, is a prototype of another class of substances (C18 phenolic steroids) which has previously unsuspected biological properties which we have found in the course of our studies. These include potent suppressive action on certain delayed-type immunologic responses such as those represented by the skin reaction to tuberculin and thyroglobulin; as well as those organ responses in which delayed-type sensitivity is considered to play a prominent role, such as auto-immune thyroiditis and adjuvant-induced immune polyarthritis. The close morphologic and histologic resemblance of immune

(adjuvant) polyarthritis in rats to human rheumatoid disease, and the marked suppressive action of estriol on the former, have prompted its use in large amounts in therapy of rheumatoid patients and the results are clearly beneficial; an extensive clinical trial appears desirable. Estriol and its natural and synthetic congeners have also been shown to markedly impair the capacity of the liver to excrete sulfobromophthalein (BSP) into the bile. The mechanism of this steroid action has been examined in detail in man and experimental animals; its structural basis has also been established. This estrogen effect applies equally to hepatic disposal of bilirubin and probably also to drugs such as tetracycline, for which the liver represents a major excretory pathway. An estrogen action of this type undoubtedly accounts in large part for certain hepatic excretory impairments which characterize pregnant women, neonatal infants, and of particular interest, women who use the new contraceptive pills containing synthetic hormones.

A variety of other studies, principally metabolic in type, is also in progress. These include investigations on the capacity of estrogens to counteract the chemical derangements accompanying hyperparathyroidism in man; the effects of estradiol and estriol on hydroxyproline metabolism in humans; the effects of these steroids on secretory rates of cortisol and aldosterone and on production of cortisol and thyroxine-binding globulin (with

particular emphasis on the principal basis of these actions) and the nature, source and role of hormonal substances in parotid gland secretions.

Radiation Injury

G. V. LeRoy, J. H. Rust and G. B. Ho

It is known that radiation injury is associated with alterations in a number of biochemical processes, such as biosynthesis of nucleic acids, metabolism of sulfhydryl-containing compounds, biosynthesis of cholesterol, increased glycogenesis, et cetera. None of these changes, however, seems drastic enough to be lethal either individually or in concert. Seeking a biochemical lesion caused by a lethal dose of radiation, we investigated some aspects of the intermediary metabolism of simple carbon compounds that are oxidized for energy production. When we looked for evidence of inactivation of enzymes in intact animals we studied the time-course of $^{14}CO_2$ in expired air after administration of certain substrates (bicarbonate, formate, acetate, pentose and hexoses) labeled with radiocarbon. In addition, we examined in a preliminary fashion the fixation of CO_2 in liver glycogen, and the transfer of carbon from various sources into urea.

The results of the first series of experiments -- using nearly 1,000 rats -- can be summarized:

(1) There is a significant decrease in the output of CO_2 in expired air: the respiratory quotient becomes less than 0.7.

(2) The apparent rate of oxidation of pentose, hexose, and acetate, as measured by the appearance of $^{14}CO_2$ in expired air, decreases to about 3/4 of the value in controls: this cannot be attributed to injury to intracellular oxidative enzyme systems.

(3) The metabolic pathway for the transfer of carbon from alanine (pyruvate) to urea was not sensitive to radiation, whereas that for glucose carbon was seriously impaired.

(4) Glycogenesis by the liver was significantly increased.

(5) Fixation of CO_2 in liver glycogen was more than 70 times greater in the irradiated rats than in the controls.

It seems that radiation may have a greater effect on anabolic processes -- which it appears to enhance -- than on catabolic or oxidative reactions. Further studies of the enhanced glycogenesis are in progress.

Respiration Pattern Analysis

G. V. LeRoy

Respiration pattern analysis is a relatively new technique for the in vivo study of biological systems. Analysis of the rate of expiration of $^{14}CO_2$ following administration of an appropriately labeled substrate provides a powerful tool for the study

of metabolic pathways and for examination of individual variations in different metabolic states and disorders with little or no disturbance of the subject. The early work in this field began independently about 12 years ago at the Donner Laboratory (University of California) and here at the Argonne Cancer Research Hospital. We use a GM detector in our instrument, while most other workers use an ionization chamber system. Our instrument was the first to incorporate a complete information-logging and processing system, thus permitting machine analysis of data.

We have devoted a great deal of effort to studies of the CO_2 pool in man since it is the final common pathway through which $^{14}CO_2$ of metabolic origin must pass. Although dimensions can be assigned to the pool there are good reasons to question their validity. Doubt arises because of the need to assume the existence of a steady-state during an isotope dilution experiment. It is debatable if this is a valid assumption for short-term periods of observation in man.

It appears that it may not be necessary to know the dimensions of the CO_2 pool for many applications of respiration pattern analysis.

The Argonne Cancer Research Hospital' Total-Body Counter

R. J. Hasterlik, G. V. LeRoy and C. M. Newton

Metabolic studies using calcium-47 as a tracer of calcium metabolism have been completed on 16 hospitalized patients. In

addition, studies of the metabolism of real and simulated fission products have been carried out on 102 healthy volunteers.

In order to quantitate changes in body content of a radioisotope with the greatest precision, it is necessary to design the facility with maximum flexibility for crystal arrangement and then to arrange the crystals in their optimally determined positions. Studies carried out in the past 2 years have been concerned specifically with 2 factors contributing to the insensitivity of the counters to redistribution of an isotope in the body. (1) The crystal array may count an isolated source with an efficiency sensitive to source position. (2) Distortion of the observed γ spectrum by Compton and other absorptive effects is sensitive to body build and isotope distribution.

We have written a computer program for the University's IBM 7094 which expedites determination of the optimal 2-, 3-, and 4-crystal linear arrays for any specified linear source locus parallel to the array axis. Degree of optimization for any crystal array is estimated by an error function, and the calculated error is printed for crystal positions near the optimal.

A method has been developed for approximate correction for counting efficiency shifts resulting from distortion of the γ spectrum within bodies of varying size and shape. A standard radioactive source was taped to selected points on the surface of a subject's body and the subject counted prone and supine.

Averaged prone and supine spectra provided data for a regression calculation of constant coefficients. Using these coefficients, the formula's ability to correct for shifts in photopeak counts was then tested by counting the same patient at certain times after ingestion of a nonabsorbable capsule containing a known quantity of the same isotope.

Inspection of data derived by these methods leads to the conclusion that the described methods do indeed provide a desired correction. These studies are preliminary to the development of methods for the derivation of corrections for accurate quantitation of two γ-emitting radioisotopes counted simultaneously in individuals of differing size and shape and which may translocate during the course of the studies.

Studies of Real and Simulated Fallout

G. V. LeRoy, J. H. Rust and R. J. Hasterlik

Real and simulated particulate fallout and solutions of strontium-85 chloride and cesium-134 chloride were fed to 102 healthy volunteers. Absorption and retention of ingested radioactivity were measured by whole-body counting using the gamma-ray spectrometer that was constructed for the Argonne Cancer Research Hospital. An average of 3 per cent of the γ-radioactivity of week-old local fallout was absorbed. The doses fed were too small to permit estimates of rate of elimination or identification of

particular nuclides. Using simulants and solutions of ^{85}Sr and ^{134}Cs, useful information was obtained on the distribution of values for absorption and retention.

The average absorption of strontium was 16 per cent, and the range was 8 to 34 per cent. Excretion of strontium varied considerably: the median value for retention at one year was estimated as about 16 per cent, and the range was from none detectable to about 25 per cent. The metabolism of strontium was the same when it was given as a solution of chloride, or leached slowly from simulated fallout.

The biological half-time for excretion of cesium was 91 ± 18 days. About 90 per cent of the material was absorbed when it was fed as a solution of cesium-134 chloride.

Absorption and retention of barium was as variable as that of strontium. When ^{133}BaO was fed, absorption ranged from 1 to 15 per cent.

Studies on the Metabolism of Magnesium in the Rat

J. G. Chutkow

Previous work on the absorption, excretion and tissue distribution of magnesium in normal animals using ^{28}Mg has been extended to young rats fed a diet low in magnesium. Symptomatic magnesium deficiency was accompanied by hypomagnesemia; hypophosphatemia that could be corrected by realimentation with magnesium; continued

fecal and negligible urinary excretion of ^{28}Mg; and an increased absorption which was not due either to hypomagnesemia *per se* or to a selective increase in uptake of magnesium in any one segment of bowel. These absorptive changes could be reversed initially but not later by large amounts of magnesium administered by gavage.

In normal bone, kidney, heart, and liver, exchangeable magnesium appeared to be in at least 2 forms, one of which turned over more rapidly than the other. Brain, muscle, and testicle took up ^{28}Mg slowly and lacked the more labile phase. The radioisotope was diverted from bone to the soft tissues in hypomagnesemic rats.

Experiments on the central nervous system, renal and cutaneous effects of magnesium deficiency are under design at present. These will include tissue electrolyte and light microscopy studies. The kidneys will be examined by electron microscopy. In addition to the electroencephalographic changes accompanying the development of the audiogenic seizures in magnesium deficiency, possible alterations in the content and distribution of serotonin and norepinephrine in the brain will be studied. Similar chemical determinations will be made on the skin during the phase of intense vasodilatation.

Specific Metabolic Processes in Skin

A. L. Lorincz

It was recently demonstrated in this laboratory that normal appearing skin surfaces in psoriatic patients have only one-tenth the cholesterol esterifying ability shown by skin surfaces of non-psoriatic persons. There is reason to believe that this epidermal biochemical deficiency related to disturbed keratinization may represent the basic, genetically determined, defect underlying susceptibility to psoriasis. Experiments are currently underway using ^{14}C acetate incubated with epidermal sheets to show by means of thin layer chromatographic techniques the precise ways in which cholesterol producing metabolic pathways differ in normal-appearing skin of psoriatic subjects from such pathways in skin of normal subjects. Further studies on skin surface cholesterol esterifying ability in the kindred of psoriatic patients are also being initiated to determine the mode of inheritance of the deficiency shown by psoriatics, and to see whether latent susceptibility to psoriasis can be detected.

Additional studies to develop a means for quantitatively measuring the rate of physiological desquamation under normal and various disease conditions using radiosulfur-labeled cysteine tracer techniques are being pursued. The rate of physiological desquamation is believed to have critical bearing on susceptibility to a number of skin diseases and infections.

PROBLEMS IN SCANNING

A Theory of Radioisotope Scanning Systems

R. N. Beck

The principal goal of a general theory of scanning systems is to enable one to predict and evaluate the performance of hypothetical systems, optimally designed for specific scanning applications; e.g., brain tumor detection. Such a theory should provide quantitative answers to such questions as "How does a scanning system designed for ^{131}I radiation compare with one designed for ^{203}Hg in detecting brain tumors of a certain size, depth, etc.?"

This paper attempts to organize the various components of such a theory, and to derive equations which relate the biological and physical parameters that must be considered. These include tumor size, depth, uptake ratio, collimator sensitivity, resolution, focal length, scan area, time, reliability, etc.

Central to such a theory is a criterion or figure of merit which can be computed for any system and used to compare different systems. Criteria based on statistical considerations and information theory are derived. In both cases, these are functions of the sensitivity and spatial resolution of the collimated scintillation detector. Collimator resolution, as defined by some fraction of the width of the point source response curve, is

inadequate for predicting the response to a distributed source. An analogous situation exists in optics where "it has been increasingly realized that the advantages of resolving power as a criterion of quality are largely illusory." Borrowing from that field, the concept of "sine wave response" is introduced to generalize the definition of resolution of collimated scintillation detectors for distributed sources. The system figure of merit is then expressed in terms of detector sensitivity and sine wave response.

Collimator response to point, plane, and volume distributions of radioactivity is discussed in detail. The total response $\left[\bar{E}_t = E(1 + P + S)\right]$ of a collimated detector viewing a large distributed source consists of 3 components produced by gammas which enter the collimator 1) "geometrically" or properly (E), 2) by penetrating the collimator septa (EP), and 3) by scattering in the source or collimator (ES). Exact equations for these components are very complex for multichannel collimators. Useful approximate expressions are derived for E, P, and S, and the limitations of these expressions are discussed.

Collimators for Radioisotope Scanning Systems

R. N. Beck

The total response of a collimator viewing a large distribution gamma ray source consists of 3 components produced by

gammas which enter the collimator 1) "geometrically" or properly, 2) by penetrating the collimator septa, and 3) by scattering in the source or collimator. This paper describes procedures for the design, construction and testing of focused collimators based on equations for these components. Since no single design procedure is appropriate for the entire range of gamma energies, the problem is considered in three parts.

1) Below approximately 0.150 MeV, few gammas can penetrate the thinnest lead septa which can be conveniently cast. Here the design procedure maximizes geometrical response for specified focal length, radius of view, septum thickness, and crystal diameter (or "shape factor," which determines the divergence of the collimator field of view, and can be used as an independent variable in place of crystal diameter).

2) In the energy range from approximately 0.150 to 1 MeV the response to gammas which penetrate the collimator septa is not negligible and must be controlled. In this case, geometrical response is maximized for specified gamma energy, collimator material, focal length, radius of view, penetration fraction and crystal diameter (or shape factor).

3) Above approximately 1 MeV it is not always possible to design a multichannel collimator having acceptably small penetration. In this case, a single hole having a taper which maximizes geometrical response is used.

Techniques for casting and "facing off" multichannel collimators having very thin septa (approximately 0.007 inches) are briefly described.

Following modern practice in optics, a procedure is described for measuring collimator resolution in terms of "sine wave response" using a "sunburst" test pattern. It is suggested that this pattern be adopted as a standard for measuring overall system resolution for distributed sources.

Techniques Which Aid in Quantitative Interpretation of Scan Data

D. B. Charleston, R. N. Beck, P. Eidelberg and M. W. Schuh

This paper discusses a range of techniques which assist in the evaluation and interpretation of scanning readout display. The range extends from simple interval calibration for photographic readout, to fairly elaborate auxillary equipment for presentation of accumulated digital scan information to a computer program.

The direct and remarkably useful method of using a random pulse generator to produce a calibrated step-wedge of spots which is projected onto a film by the same projection light source as is used during the scan, allows the viewer to compare exposure densities of regions of interest on the scan to similar regions on the wedge which are calibrated directly in count rate units.

Auxillary equipment, such as a multichannel analyzer used in the multiscaling mode, permits the accumulation of digital information for a total "count per scan-line" display for each index step.

Small animal scans have been made which accumulate and display "counts per line scan" for each index step. This produces an accurate quantitative measure of the distribution of activity over the animal, and a profile display of activity similar to the slit scan display of a linear scanning system.

The same multiscaling technique is carried further by accumulating digital information for a "count per unit area" display. A profile curve is obtained for each scan line of each index step. From this it is possible to visualize or construct an area profile of count rate.

Precise position information must be included with the data record.

Computations of per cent difference of activity in regions of interest on opposite sides of the head, are made from data accumulated by multiscaling, for use with the Argonne Cancer Research Hospital's brain scanning system.

Collimators for Gamma Ray Cameras

R. N. Beck, P. V. Harper, E. Schmidt and L. T. Zimmer

An image of distribution of radioactivity can be produced not only by scanning systems, but also by fixed devices called gamma ray cameras. Recent acquisition by the Radiology Department of an Anger-type camera has stimulated interest in the design of collimators of high Z material which perform the function of a lens for such devices. The collimator may be a "pin hole" (for relatively thin sources, e.g., the thyroid) or an array of many holes covering the entire crystal face (for large thick sources, e.g., the brain).

The goal of multichannel collimator design is to maximize the collimator sensitivity for a specified gamma energy resolution and negligible penetration. The appropriate design procedure is determined by the proposed method of construction. Equations have been formulated and a design procedure developed for collimators consisting of cylindrical holes to be made from extruded lead tubing or tubing rolled from lead foil. An alternative set of equations and design procedure applies to collimators consisting of tapered holes with parallel axes, to be cast in lead. The IBM 7094 computer has been programmed to carry out the design procedures for both types of multichannel collimators for any specified gamma energy, crystal diameter, resolution and penetration fraction.

Equations have also been formulated for pin hole collimators, showing that geometrical efficiency can always be made comparable with multichannel collimators, for the same crystal diameter and resolution. However, these equations also show that pin hole collimation will be most effective in scanning small sources or with small detectors.

Response of Scintillation Detectors to Scattered Radiation

R. N. Beck and M. W. Schuh

An adequate theory of scanning systems must enable one to compute the total response of collimated scintillation detector to a volume distribution of radioactivity. This consists of the sum of responses to those gammas which enter the collimator "properly," those which enter the detector after penetrating the collimator or shielding material, and those which enter as scattered radiation. Previous papers have dealt with the first two components.

To evaluate the scatter component it is necessary to determine the fraction of counts within the photopeak due to gamma rays which have been scattered in the source or collimator. To accomplish this it is necessary to know the shape of the scatter spectrum within the photopeak. This can be determined by properly smearing the Klein-Nishina equation for the scattered photon energy

spectrum. The IBM 7094 computer has been programmed to carry out this procedure and to compute the scatter fraction as a function of base line setting.

Although pulse height analyzers are ordinarily used to reduce the deleterious effects of scattered radiation, the problem of selecting a base line setting which achieves an "optimum" compromise between the recording of scattered and unscattered radiation has not been adequately discussed. Treating scattered radiation as "noise" or "background," an optimum base line setting can be found for which the error in measuring the number of pulses due to unscattered gammas is minimum. This procedure has been carried out for several gamma emitters in a brain phantom for the energy range commonly used in brain scanning, 140 to 510 keV.

A Precision Scanning System Employing Digital Drive and Digital Control Techniques

D. B. Charleston, R. N. Beck and J. C. Wood

Careful analysis of medical isotope scanning system readout presentations (usually photographic) has indicated that even small irregularities in the mechanical scanning motions can cause distortion (mechanical modulation) of detected information which will be superimposed upon the readout. These irregularities enhance or obscure regions of interest within a readout and can contribute

to a visual misinterpretation of the actual isotope distribution. With the advent of fast scanning techniques with inherently good spatial resolution capability, it becomes increasingly important to eliminate degradation of the readout by mechanically produced artifacts.

A mechanical scanning system incorporating pulsed stepping drive motors for both the sweep and index motions presents scan data accurately, without distortion due to mechanical modulation. The sweep speed can be controlled by the drive pulse rate. A stable multi-range pulse generator assures a wide range of precise sweep speeds. Two manual digital numeric settings on a "foreward-reverse" preset counter establish the scan sweep limit. A pre-selected digital setting also selects the scan index-step increment.

It is possible to use a digital drive scanner in a fixed-count-per-unit-area mode of data presentation as opposed to the conventional fixed-time-per-unit-area mode. Conventional scanners operate in the constant time-per-unit-area and record detected events as a variable. With digital scanning, the detected events may be used to drive the scan motor (speed dependent upon count rate) which assures a fixed number of counts per unit of scanned area while time is recorded as a clock pulse. This novel technique offers the advantage of presenting uniform counting statistics over the entire scan area.

A digitally controlled system has advantages over other scanning systems in that all drive pulses can be utilized externally as time and position signals for data storage or direct computer use; remote or parallel readout systems can be synchronized precisely with the basic drive pulse; operation and set-up of the scanning system is considerably simplified, and the internal electrical noise generation usually present in most mechanically switched devices is eliminated.

Resolution Versus Sensitivity in Scanning

P. V. Harper and R. N. Beck

In an effort to develop a method for optimizing scanning parameters, and particularly to find a rational way of compromising between sensitivity and resolution, a series of Brownell's synthetic scans was shown to 10 individuals who were instructed to score as 100 per cent the picture which best represented the object, and to rate the other pictures between 100 per cent and 0 per cent according to their best judgment. The object chosen was a square field containing at its center a disc one-half the diameter of the field with a 5 to 1 concentration of activity over the surrounding area. As expected, the scores were low at very fine resolution where there were very few counts and much statistical fluctuation, and at very coarse resolution where the edges of the disc were poorly defined. Since the object contained a

single sharp contour, it seemed appropriate to maximize the slope of the gradient of the information density across this contour. In crossing the contour, if there were infinite time to count, the average true means count rates would be known and the difference of the average counts per resolution area on both sides of the contour would be $N_o(R-1)$ where N_oR = counts/resolution area in the disc, N_o the counts/resolution area in the surrounding region, R in this case being equal to 5. The uncertainty removed by making this measurement would thus be $N_o(R-1)$, and the information gained in natural units $I = \ln N_o(R-1)$. Since however the scanning time is limited, there is a residual uncertainty due to statistical fluctuation whose σ is $\sqrt{N_o(R+1)}$. The information associated with this uncertainty is $\ln \sqrt{2\pi e\, N_o(R+1)}$ and thus the information gained in moving the detector across the contour is

$$I = \ln N_o(R-1) - \ln \sqrt{2\pi e\, N_o(R+1)} \text{ or } 1/2 \ln \left(\frac{N_o}{2\pi e} \frac{(R-1)^2}{R+1} \right)$$

Since the length of the gradient across the contour is approximately the resolution length, d, the information gradient

$I' = \frac{1}{2d} \ln \frac{N_o}{2\pi e} \frac{(R-1)^2}{R+1}$. In order to optimize this expression it is necessary to express N_o in terms of d. For a moving collimator system with a focused collimator and constant crystal diameter the sensitivity is proportioned to the area of view. Similarly the time spent looking at any one area is proportioned

to the area of view, scanning times being equal. N_o therefore $= Kd^4$ where K is a constant. I' then $= \frac{1}{2d} \ln \frac{Kd^4}{2\pi e} \frac{(R-1)^2}{R+1}$. Differentiating I' with respect to d and equating to zero gives N_o (optimal) equal to $2\pi e^5 \times 3/8$ or 349 counts/resolution area. This differs radically from the experimental situation where N_o opt $\approx$ 18. This divergence disappears however when one considers that in viewing a contour the eye does not look at a single pair of resolution areas on opposite sides of the contour but at a number of these distributed along each side of the contour. The number of counts/resolution area thus becomes averaged over a number of resolution areas and if we choose this number, b, as $\frac{349}{18} = 19$ it does not appear unreasonable. Thus we obtain an expression that describes the experimental results rather closely, i.e., $I' = \frac{1}{2d} \ln \left(b \frac{N_o}{2\pi e} \times \frac{R+1}{(R-1)^2} \right)$, when I' is normalized against the "optimal" picture scan as judged by observers.

A 4π Animal Counter Using Plastic Scintillators

D. B. Charleston and N. J. Yasillo

The need for a rapid counter for <u>in vivo</u> measurement of radioactivity in animals which is independent of both the activity distribution within the animal and the animal size (rabbits and smaller animals), led to the design of an approximate 4π counter.

Unique features of this design are found in the well configuration, the two-piece plastic scintillator, the photomultiplier tube placement, the animal loading method and the lead shot shielding.

The detector consists of two identical NE-102 scintillating plastic cylinders 17 inches in diameter, 14 inches long, each with a six-inch diameter well. The wells are designed to have a straight cylindrical section six inches deep with a hemispherical bottom of a three-inch radius. The two sections of scintillator close mechanically, with the wells facing, to form a well "capsule" 18 inches long and six inches in diameter. The animal is placed in a container of the same shape as the well capsule which is inserted into one part of the split counter; the other counter section closes to envelop the container. The animal is thus surrounded with a minimum of four inches of plastic scintillating material.

The opposite flat surfaces of the scintillators have 7 five-inch photomultiplier tubes attached, one centered and six equally spaced around it. The shielding consists of welded hollow steel containers. Fine lead shot is poured into the containers to complete the shielding after the unit is installed. The lead shot offers approximately 85 per cent as much shielding as solid lead and can be drained from the containers if the unit is to be moved.

With the split scintillator design it is possible to construct a counter with optimum geometry (4π). Conventional annulus counters not only have a lower geometry factor but reduce the

possible light collection efficiency. (Fewer tubes can be utilized at the light collection surface due to the concentric opening.)

The spherical shape of the well ends allows each photomultiplier tube to "see" more of the total scintillator volume than is possible in the more conventional right-cylinder well shapes. The individual sections of the split scintillator present a better aspect ratio (ratio of length to diameter) to the light collection surface. Both features contribute to better spectrum resolution.

Modification of the ACRH Brain Scanner

R. N. Beck, D. B. Charleston, P. Eidelberg

and P. V. Harper

The ACRH brain scanning system consists of four scintillation detectors, arranged in opposing pairs, which scan both sides of the patient's head simultaneously. These detectors are housed in lead shields which accept interchangeable focused collimators designed to give maximum counting efficiency for specific gamma energies. Detected gamma pulses are fed to a transistorized electronics unit consisting of four pulse height analyzers together with pulse forming circuits for photographic recording on two sheets of film, one for each side of the patient's head. Photopeak gamma pulses from each channel produce bell-shaped spots on film by means of pulsed light projectors.

For each channel, a calibration step wedge is produced on the film record by a random pulse generator which produces count rates of 100 to 9500 counts per minute in twenty-six steps of 20 per cent increase. This permits a quantitative measure of the count rate at any point on the patient's head.

Small lights in the center holes of collimators on one side are focused on photodiodes in the opposite collimators. Signals produced by this system outline the patient's head on the film record and reverse the scan direction to minimize the time spent in scanning beyond the head. A dual channel optical system records data relating to the scan (patient's name, isotope injected, scan speed, etc.) on each film record together with an appropriate LEFT or RIGHT mark for unambiguous identification of the film.

Using ^{99m}Tc and a scan speed of 2.5 cm/sec., this system produces pictures of conventional quality of both sides of the patient's head in 90 seconds. If the scan time is extended to 15 minutes, the scan pictures are decidely improved.

As originally designed, these detectors were shielded by 2" thick lead, adequate for gamma energies up to that of ^{131}I (364 keV). The weight of these shields (approximately 275 lbs. each) limited the maximum safe scanning speed to 1" per second, while their physical size limited the brain area which could be conveniently scanned in patients with short necks.

For gamma energies below 200 keV, adequate detector shielding is obtained with much less lead. Shields consisting of 1/2" thick lead have been constructed to replace the more massive shields. These are mounted so that the separation can be conveniently varied from 8" to 14-1/2". Thus the device can be used with low energy gamma ray emitters for liver, lung, kidney and thyroid scanning, in addition to brain scanning. Collimators have been designed for each of these procedures.

Modification of the patient cot has been undertaken to facilitate patient handling. The scan motor is to be replaced with an impulse motor to achieve a wider range of stable scan speeds.

Modification of the Picker Magnascanner

R. N. Beck, D. B. Charleston and P. V. Harper

The collimator-detector assembly of the Picker Magnascanner was designed for use primarily with gamma emitters in the energy range between 200 - 500 keV. To improve the sensitivity and resolution at lower energies, an auxiliary, light weight, low energy probe has been attached to the Picker detector carriage. This detector consists of a 1.75" diameter X 0.25" thick NaI (Tl) crystal with a beryllium window and an EMI 2" photomultiplier tube. Three interchangeable collimators having 253 tapered holes provide resolutions of 0.156", 0.218" and 0.314". To maximize collimator efficiency, septum thickness has been reduced to approximately 0.010", which is adequate for gamma energies up to approximately 140 keV.

Pulses from this detector can be routed through the Picker electronics circuits and recording system, or through an auxiliary amplifier-pulse height analyzer and light projector which operate independently of the Picker system. The auxiliary light projector produces a bell-shaped spot on film for each detected gamma. These spots overlap to form a smooth image of the distribution of radioactivity with a minimum of "spot structure." In addition, the scanning speed has been increased to 3" per second so that the space between scan lines can be reduced. This has the effect of reducing the recorded "line structure." The overall effect is a significant improvement in picture quality for a given scanning time and radiation dosage to the patient.

Modification of a Laminated Iron Room by the Addition of a "Drawbridge" Type Patient Transfer Assembly for Whole-Body Scanning

D. B. Charleston, E. Mason and J. J. Stupka

A safe transport device for positioning non-ambulatory patients in a whole-body counting cell is a necessity for biological and medical research in a hospital. A mechanical system has been designed to serve as a patient transfer cot and/or the transport mechanism for a whole-body linear scanner.

In the scanning mode of operation, a subject is moved slowly into the iron room past fixed multiple detectors which

have slit or focused collimators. In this way a plot or data record of the radioactivity distribution over the length of the subject is produced.

The "drawbridge" system design lowers the portal shielding (lead) through a 90° arc, from the upright or portal shield position over the opening in the face of the iron room, to a horizontal position. The shield then becomes part of a support table for the patient transfer cot. After the subject is moved into place within the cell for whole-body counting (no scan), the "drawbridge" shield is raised back into the portal shield position until the count is completed. Since the detector within the cell will have additional shielding and collimation when operating in the scan mode, the shield can remain in the open position (horizontal) during the scan. Negligible background contribution is noted in the scanning configuration.

The "drawbridge" design was selected because it has the following advantages:

Safety: No hinged or sliding doors interfere in the patient handling area. The shield mass is positioned under the patient cot.

Size: When not in use, the device is in the upright position at the face of the cell. The external drive mechanism occupies minimal floor space outside the cell. The system does not interfere with the interior placement of crystals or equipment.

Versatility: The transport mechanism doubles as a patient transfer cot and a scan drive mechanism. The addition of the "drawbridge" system does not alter the whole-body counting capability of the iron room.

Economy: The portal shield becomes a support table and part of the drive mechanism (reducing the amount of construction and structure). The same instrumentation and equipment (including detectors) can be used to perform both the scanning function and the whole-body measurements; additional electronic equipment is not necessary. Additional space and work area are not required.

Behavioral Indicators of Small or Transient Lesions in the Nervous System

L. T. Zimmer

Routine methods of pathology do not show changes in the adult central nervous system (CNS) at radiation levels which induce easily detectable changes in other cell systems. Furthermore, morphological or chemical changes in individual cells of the CNS, due to normal plasticity in behavior of the whole organism, are exceedingly difficult to find even though these behavioral changes may be massive and obvious on a macroscopic level. These facts suggest that organism (system) behavior may be a more sensitive

detector of the effects of low radiation levels than individual cell behavior, and recent evidence from both American and Russian sources tends to confirm this. Altered macroscopic behavior of an organism may be regarded as a sensitive biological amplifier of subtle CNS change; it also indicates how those CNS changes are important to the organism in relation to its external environment.

An analogous situation exists in neurophysiology: application of an appropriate stimulus to the brains of lissencephalic animals induces a transient loss of CNS function known as "spreading depression." Several measurable quantities (electrical, vascular, metabolic, etc.) disclose the temporary effect, though none indicate a permanent change. At the same time, by manipulation of environmental factors, relatively permanent modifications of behavior may be induced, characteristic of the state of the CNS during the reversible lesion. Such behavioral modifications, when correlated with altered functional properties of the CNS as deduced from the measured physiological changes, provide information about the neural basis of behavior. In addition, the feasibility of behavioral methods for indicating small CNS changes is demonstrated.

Work is in progress, using chronic rat preparations, 1) to develop behavioral situations with optimum sensitivity to the effects of cortical spreading depression and, 2) using a technique developed here which continually monitors brain electrical

activity in an unanesthetized and unrestrained rat, to measure detailed electrical changes resulting from spreading depression simultaneously with measurement of behavioral responses.

Technetium-99m as a Scanning Agent

P. V. Harper and K. Lathrop

The physical characteristics of ^{99m}Tc -- its 6-hour half-life, clean 140 keV gamma, and absence of particle radiation (except for conversion electrons) make it ideal for clinical use from a dosimetric point of view since it is available as the daughter of the 2.8 day molybdenum-99. Large doses of the isotope give trivial radiation dosage (1 mc evenly distributed gives approximately 10 m rad total-body dosage). Since up to 2 per cent of the injected dose is trapped in the gland in euthyroid patients, the localization of TcO_4^- in the thyroid gland permits scanning when 1 to 2 mc are administered. This trapping is increased by a large factor in hyperthyroidism, so that hot nodules are particularly well demonstrated by this technique. Pertechnetate also permits visualization of the salivary glands and stomach although no great clinical use has been made of this. Intrathecal TcO_4^- disappears with great rapidity ($T_{1/2} \approx 2$ minutes), but after pretreatment with ClO_4^-, which paralyzes the concentrating (deconcentrating) mechanism of the choroid plexus, the activity stays in the subarachnoid space for hours and may be used

for cysternography or myelography. Plasma albumin tagged with ^{99m}Tc may be used for blood pool scanning, and, when aggregated, for lung scanning. Technetium sulfide carried on sulfur colloid behaves like colloidal gold, and may be used for liver, spleen, and bone marrow scanning. As the thiocyanate dissolved in fat emulsion, ^{99m}Tc is localized in the polygonal cells of the liver. As the glycine complex and ferric hydroxide complex, it is excreted rapidly by the kidney and may be useful for renal scans. The very high activities which may be used result in greatly improved collimator resolution and scanning speed. The moderate energy of the gamma permits very efficient shielding, collimation, and detection with light weight probes. The cost and availability are reasonable.

The Pharmacodynamics of Technetium Pertechnetate ($^{99m}TcO_4^-$)

P. V. Harper and K. Lathrop

Pertechnetate behaves in the body in a manner similar to iodide, ClO_4^-, ReO_4^-, BF_4^-, At^- and, to a lesser extent, a number of similar ions. Initial distribution is in the extracellular spaces with trapping by the stomach, thyroid, and salivary glands, and exclusion from the spinal fluid by the choroid plexus. This localization can be prevented by administration of sufficient amounts of perchlorate or iodide, presumably by competitive inhibition or saturation of the localizing mechanism.

The blood disappearance curve following intravenous administration resembles that of iodide, although a somewhat higher fraction of the injected dose is carried in the plasma due to loose protein binding, which is somewhat greater than with iodide. As time goes on, the Tc activity is localized to an increasing extent in the liver and intestinal tract, presumably due to reduction of the TcO_4^-. This is reflected in the excretion pattern. In human beings, about half of the Tc is excreted in the urine, most of it on the first day, and about one-third is excreted in the feces during the second and third days after administration.

In mice the initial localization of 30 per cent of the injected dose in the stomach makes this the critical organ from the point of view of dosimetry. The thyroid localization in mice is reduced by administration of thyroid hormone, and not greatly increased by TSH or propylthiouracil. The T/S ratio is about the same as for iodide, so that TcO_4^- is a fair indicator for the activity of the thyroid trapping mechanism. In human studies hyperthyroid glands have greatly enhanced trapping of pertechnetate which is easily washed out with perchlorate.

^{131}I-Antifibrinogen

P. V. Harper and I. Spar

We have been working in collaboration with Bale and Spar of the University of Rochester on the use of ^{131}I-antifibrinogen as

a diagnostic and possibly therapeutic agent. This material presumably combines with circulating fibrinogen and is carried down in regions of the body where fibrin is being deposited, as in a tumor causing a reactive inflammation in its stroma or the surrounding tissue. In approximately half of a wide variety of tumors, localization was sufficient to permit clear visualization on scanning, and in several of these was sufficient to justify administration of a therapeutic dose. One method of enhancing the localization is by administration of epsilon-amino-caproic-acid to inhibit fibrinolysis and prevent mobilization of deposited activity. Another method is to irritate the tumor to increase fibrin deposition.

Removal of the residual circulating activity to reduce the background in the case of scanning, or the total-body radiation in the therapeutic situation, is possible by administration of an antibody to the antibody. An amount of goat anti-rabbit gamma globulin equivalent to the amount of rabbit anti-human fibrinogen (about 0.1 mg) is administered intravenously. The antibody antigen reaction takes place in the circulating plasma, and the antibody antigen complex is removed from the circulation in the liver with a half time of approximately 6 hours, metabolized, and the iodine-131 excreted. This procedure has been carried out in 2 patients, of whom one, having only a suspected tumor, excreted the iodine rapidly in 48 hours. In the other, who had an extensive

tumor, there was a rapid disappearance of the activity from the circulating plasma but the total-body activity disappeared much more slowly, approximately 20 per cent per day, suggesting that the tumor was holding antibody to fibrinogen.

It is our feeling at the present time that this study, while of interest, is not a substantial help in the management of malignant disease, because of the rather unpredictable uptake and because the therapeutic possibilities seem too meager to justify broad screening.

Short-Lived Isotopes

P. V. Harper, K. Lathrop and E. Schmidt

Short-lived isotopes are available to us from 1) a longer-lived parent, 2) reactor 3) cyclotron or 4) linear acceleration (γ-n). Although these are the most common and flexible sources, reactor- or cyclotron-produced isotopes must have half-lives of several hours to be of much use at this Institution because of transportation problems.

Perusal of the existing parent-daughter pairs reveals two whose possible medical uses have not been explored, ^{144}Ce (280 d) - ^{144}Pr (17 min, 98 per cent 3.5 MeV β) and ^{113}Sn (112 d) - ^{113m}In (1.7 hr, 390 keV γ). Attempts are currently in progress to devise methods of separating these daughter isotopes into forms that might be useable medically. The 17-min Pr would be most suitable for flow studies using an intravascular detector. In

such studies that use phosphorus for instance, prohibitively large amounts of isotope must be given. Another possible use might be therapeutic irradiation of the gastric mucosa, perhaps even without enclosing the isotope, since with carrier there is little absorption and thus little distant and no long-term radiation. The Sn-In pair has also been separated, though not completely, in a resin column and its chemical parameters are being explored. The possible uses of the indium are completely unknown. The early localization has not been studied.

Accelerator-produced isotopes exist in wider variety. As an example, the present Argonne Cancer Research Hospital linear accelerator, using 25 MeV electrons with a beam current of 0.02 μα and a one-eighth inch converter plate of lead, produced 200 μc/gm of 7-minute potassium-38. With the beam current and energy increased, the yield may be improved by a factor of 100 or more. ^{38}K should be an ideal agent for coronary flow measurements. Since it is a positron emitter, detectors can be designed to give almost completely uniform sensitivity in the region of the heart without absorption or collimation effect. The sensitivity can thus be calibrated directly, and the fraction of activity remaining in the myocardium after the first recirculation should approximate the fraction of the cardiac output going through the coronaries. In such a system, accelerator-produced chlorine-34 would be used to measure extracellular space, and ^{124}I

albumin to measure the intravascular spaces. Such possibilities give glimpses of vistas which support the consideration of a small cyclotron and/or reactor for the Argonne Cancer Research Hospital program.

The Mapping and Display of 3-Dimensional Isotope Distributions

P. V. Harper, R. N. Beck and A. Gottschalk

The development of scanning techniques during the past several years has taken a number of directions. Theoretical progress in comparison criteria including the statistical figure of merit, modulation transfer function, and information theory formulation, has led to deeper insight into some of the problems and has permitted optimization of collimator and detector design. The development of superior scanning agents such as ^{99m}Tc and scanning modes which map the activity in a plane, as in tomography, together with the recent availability of camera-type devices, has focused our attention on approaches which were inconceivable until recently. For instance, by scanning with a camera in the transverse section mode, the entire isotope distribution in the field of view of the detector is mapped in a series of slices, giving a complete 3-dimensional representation. We are engaged currently in a study of the most efficient and economical approach to the reduction of this data. By replicating equipment, the various

levels can be presented simultaneously as an array of section scans. It would seem more flexible however, if any plane at any angle through the object could be presented. The digital approach to this problem involves immense and expensive (or clumsy) memory equipment. An alternative approach would be to reconstruct an image by projection of pencils of parallel light from the same angles as the original scans through photographic records of scans done from many angles. Intersection of the pencils of light would now produce an image by reinforcement, as is done in the 2-dimensional display, and any plane could be read out by inserting a sheet of fluorescent material into the image space in the desired location and orientation. Whether images produced by this technique would be of adequate quality is not immediately evident, but the likelihood seems to us at this point great enough to be worth a trial.

CLINICAL AND EXPERIMENTAL STUDIES ON THE EFFECTS OF RADIATION

Impairment in Delayed Response Following Bilateral Destruction of the Dorsomedial Nucleus of the Thalamus in Rhesus Monkeys

S. Schulman

Although the cortical connections of the association nuclei of the thalamus suggest that these nuclei are closely linked functionally with the cortex, the nature of the thalamic contribution to cortical function is not understood. It has long been known that bilateral lesions of the frontal association cortex in monkeys consistently result in impaired delayed response performance. Attempts to induce similar deficits by lesions in the dorsomedial nuclei of the thalamus have previously been reported by other workers, all with negative results. The present experiments were undertaken to determine whether the lack of effect of dorsomedial lesions, which has heretofore been found, may be a consequence of incomplete destruction of these nuclei. In the present study the lesions were made by stereotaxic implantation of small β-ray sources in the thalamus, rather than by the customary electrolytic technique. The sources consisted of pellets of yttrium oxide, containing yttrium-90.

Pre- and postoperative training in delayed response and in a conditional visual discrimination test was given to 9 rhesus

monkeys. The dorsomedial nuclei were completely, or nearly completely destroyed in 3 subjects. Among the others, the degree of destruction of the nucleus ranged from approximately 50 per cent bilaterally in the animal with the smallest subtotal lesions, to 98 per cent on one side and 90 per cent on the other in the animal with the largest subtotal lesions.

All of the subjects with complete, or virtually complete, bilateral destruction of the dorsomedial nuclei showed severe and enduring impairment in delayed response performance. Among the 6 subjects with subtotal lesions, 5 showed either postoperative savings or slight impairment, and one showed marked impairment, followed by improvement with prolonged postoperative retraining. The relation between the extent of the subtotal lesions and the postoperative performance in delayed response was not a consistent one. Thus, the animal with the largest subtotal lesions showed essentially no impairment in delayed response.

All but 1 of the 9 subjects showed postoperative impairment in the visual discrimination problem. This was much less severe than that in delayed response, and all of the animals eventually established criterion postoperatively.

It was concluded that the dorsomedial nucleus is an essential component of the mechanism required for the central retention of transient events, that the nucleus exhibits a high degree of equipotentiality in regard to this function, and that only a small

remnant may suffice for normal performance. The individual variation in vulnerability to subtotal lesions remains unexplained. It is believed that the nature of the impairment in the visual discrimination task was not primarily an interference with the general set to respond differentially. There was some evidence suggestive of posterior localization within the dorsomedial nucleus in regard to the visual discrimination problem.

The Destruction of Small Volumes of Tissue with Beta Sources

P. V. Harper and K. Lathrop

A study that started out over ten years ago as an effort to achieve hypophysectomy without major surgery has blossomed into a number of experimental and clinical projects. Destruction of the hypophysis with implanted yttrium-90 sources has become a rather widely used procedure. Recently the technique was modified by the use of a strong strontium-90-yttrium-90 source applied for a short time. This was attended by considerably fewer complications and produced equally good results. The same source has been used extensively by the neurosurgeons for interruption of the pain tracts in the spinal cord (at C-2) without open operation.

Yttrium sources have also been used most successfully by the neurologists to produce experimental lesions of the thalamus in monkeys. The cardiovascular group has used them in attempts to

produce experimental coronary damage, and to produce myocardial infarcts and conduction defects. Smaller lesions have been produced with palladium-109 sources in the globus pallidus for the control of Parkinsonism, and the auditory physiologists have produced medullary lesions in cats with similar sources. The opthalmologists have studied the effect of intense β radiation dosage to the sclera using yttrium-90 sources.

The characteristic which makes these sources so favorable for the above studies is the very sharp localization of a very intense radiation field, producing destructive radiation a millimeter or less away from trivial radiation, so that the lesions are very discrete, well controlled, and circumscribed. The original photographic dosimetry using the method of Tochilin and Golden appears to be satisfactory.

Strontium Cordotomy Report 1964

J. F. Mullan

One hundred and thirty-six percutaneous strontium cordotomies have now been performed for 89 patients suffering from intractable pain, mostly due to terminal cancer. There has been no mortality in the series and 80 per cent have had satisfactory relief from their pain. Failures due to inadequate dose occurred in 10 per cent while subtotal relief of pain, unpleasant paresthesias, or some degree of weakness provided less than

optimum results in another 10 per cent. This high incidence of satisfactory results together with the complete absence of mortality indicates the definite superiority of percutaneous strontium cordotomy over classical surgical cordotomy.

The long-term results of any form of irradiation are always a source of concern and since most of our patients have had terminal cancer, there has not been a good opportunity to evaluate this aspect. We do, however, have 8 patients who have lived more than 1 year after the procedure. Three of these developed symptoms of motor impairment and 2 developed abnormal motor signs without any subjective or objective weakness. The 2 with the most marked symptoms had two cordotomies each as the first one had not produced a lesion. The total periods of irradiation were 50 minutes in one and 60 minutes in another. The other 3 had periods of irradiation of 30, 30 and 25 minutes. With experience we feel that the period of irradiation should not exceed 20 minutes when the needle is in apposition with the cord. As some of this evidence of motor impairment did not appear for 6 months or more after the cordotomy, the results of an even longer follow-up period will be of special interest.

In the last year Mr. Grotenhuis of the Minnesota Mining and Manufacturing Company, has succeeded in duplicating Dr. Harper's original strontium needle in a very satisfactory manner so that it may now be obtained on a commercial basis.

This work was reported to the Harvey Cushing Neurosurgical Society at its annual meeting in Los Angeles in April 1964; as a scientific exhibit and in papers at the VII International Neuroradiologicum Symposium in New York in September 1964, and at the International Symposium on Pain in Detroit, October 1964.

The Use of Low Energy Photon Emitters for Interstitial Therapy

P. V. Harper and K. Lathrop

Low energy photon emitters may produce gamma radiations, or fluorescent x-ray, following internal conversion or electron capture. To be useful, they should have suitable penetration and a reasonably long half-life to preclude the handling of large millicurie quantities for the production of therapeutic radiation fields, and production methods and costs must be within reach. The chemical form of the isotope poses some limitations. Ten-day cesium-131, which emits 30 keV fluorescent x-rays, must be used in sealed applicators as must 60-day iodine-125 which emits principally 27.3 keV x-rays, as does 58-day tellurium-125m (daughter of 2.7 year antimony-125). The great advantages of the low energy emitters are the ease of handling and shielding, and the localization of the radiation field. The penetration of the low energy photons through tissue, even when the half-value layer is as low as one cm, is sufficient to give a relatively uniform

radiation field in the region of the implant. Our clinical experience has been limited to this energy using palladium-103 as the radiation source. The material is produced either by proton activation of rhodium-103 or neutron activation of ^{102}Pd. Twenty-five patients with a variety of inoperable lesions have been treated with various forms of implants during the past 5 years, and significant palliation has been achieved in many cases.

A General Method for Internal Dosimetry of Objects of Arbitrary Shape

P. V. Harper and E. Schmidt

Analytic expressions for the average radiation dose to an object containing a uniform distribution of a gamma emitter exist only for a few simple shapes, and then only when attenuation is neglected, i.e., when scatter compensates for absorption. For low energy emitters where attenuation becomes significant, and for odd-shaped volumes, numerical integration must be carried out.

A simple program has been devised in which the volume is made up of square rod-shaped elements of various lengths.* A library is constructed of the interactions of such elements of

*This allows any shape to be reproduced with the desired degree of accuracy, and includes most clinical situations.

various lengths at various distances. Using such a library, the desired shape may be constructed of rod elements, and the previously determined interactions summed, thus carrying out the double integration. The interactions between the rods are set up for a given gamma energy using the observed point source dose gradient in the absorbing medium. The resulting number contains only the error of assuming that the phantom is immersed in an absorbing, scattering medium. Calculations are greatly simplified by assuming bilateral symmetry.

Dose build-up curves for a number of gamma energies are available in the literature, and measurements are in progress for low energy sources such as iodine-125, using the LiF dosimeter. This approach should be particularly suitable for organ dosimetry since the organ is immersed in the body, and the method may be applied to such problems as the β dosimetry of the mouse thyroid utilizing the β dose gradient around a point source of ^{131}I. It should be of interest to compare this approach to the classical approach using μ_{eff}, and to the results using the absorbed fraction of Ellet, Calahan and Brownell.

The Late Effects of the Deposition of Radium in Man

R. J. Hasterlik

The joint Argonne National Laboratory - Argonne Cancer Research Hospital Radium Program has carried out studies since

1948 on persons carrying a significant body burden of radium. The current set of observations, underway since 1957, has resulted in the measurement and study of an additional 250 persons. These are former dial painters (82 per cent), persons given radium as a medicament by physicians (9 per cent), psychotics at the Elgin State Hospital (7 per cent), and former radium chemists (2 per cent).

The appended tables summarize these findings. Of interest is the increasing incidence of epithelial tumors in our series. These arise in those epithelial structures which lie directly on bone. Two other generalizations are also pertinent--the relationship between increasing radium content and the number and severity of bone lesions observed radiographically and between radium content and the incidence of tumors.

Table 1

SUMMARY OF LONG TERM EFFECTS OF RADIUM DEPOSITION IN MAN

Correlation of Clinical and Radiographic Findings with Current Body Burdens of Ra^{226}

Body Content (μc)	Number Measured	Number X-ray	Radiographic changes in skeleton attributable to radium deposition					
			None	Minimal	Mild	Moderate	Advanced	(Malignant)
< 0.001	30	18	16	2	0	0	0	
0.001 -0.0031	9	7	7	1	0	0	0	
0.0032-0.0099	33	24	23	1	0	0	0	
0.01 -0.031	70	59	54	5	0	0	0	
0.032 -0.099	35	30	25	4	0	1*	0	
0.10 -0.316	32	32	22	5	1	2**	2***	(1)
0.32 -0.99	33	32	4	8	8	4	7	(5)
1.0 -3.16	34	34	2	2	5	4	21	(11)
3.2 -5.5	11	11	0	0	0	1	10****	(6)
Total	287	247						

* Further studies pending
** MsTh present in each case
*** Severe tooth changes only in one case
**** Based on films taken elsewhere in two cases

July 1, 1964

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Table 2

ANALYSIS OF MALIGNANCIES ATTRIBUTABLE TO RADIUM DEPOSITION

Case No.	Current or Terminal Radium Burden (μC)	Source of Radium	Type of Malignancy	Living or Dead
03-685	0.167	occup.	Carcinoma of maxilla	Living
03-216	0.49	med.	Osteoblastic osteogenic sarcoma, left femur	dead
03-455	0.81	occup.	Fibrosarcoma, left radius	living
03-141	0.88	med.	Epidermoid carcinoma, left mastoid	dead
03-417	0.89	occup.	Squamous cell carcinoma, right maxillary antrum	living
03-402	1.2	occup.	Fibrosarcoma, proximal left femur Epidermoid carcinoma, right mastoid	living
03-209	1.2	med.	Fibrosarcoma, right scapula	dead
03-649	1.3	occup.	Osteosarcoma of left ischium	dead
03-212	1.3	med.	Osteosarcoma of left foot	dead
03-210	1.35	med.	Osteogenic sarcoma, left calcaneus	dead
03-407	1.4	occup.	Epidermoid carcinoma, right mastoid	dead
03-214	1.44	med.	Carcinoma of left mastoid	living
03-619	1.6	occup.	Osteosarcoma, left leg	dead
03-401	2.45	occup.	Pleomorphic sarcoma, left leg	dead
03-648	2.5	occup.	Mixed carcinosarcoma of right mastoid Osteosarcomas of femur and humerus	dead
03-105	2.6	med.	Carcinoma of ethmoid or sphenoid sinuses	dead
03-201	3.2	med.	Osteosarcoma, right humerus	dead
03-120	4.7	med.	Carcinoma of mastoid	dead
03-584	6.0	occup.	Osteogenic sarcoma, right pelvis	dead
03-213	6.8	med.	Fibrosarcoma of lumbosacral spine	dead
03-675	-	occup.	Rhabdomyosarcoma, right maxillary sinus	dead

July 1, 1964

The Effect of Bone Marrow Protection on Response to Irradiation

L. O. Jacobson, E. L. Simmons, E. K. Marks and E. O. Gaston

We have seen that lead protection of lymphoid cells in the small intestine of the mouse results in maintenance of the homograft reaction when the remainder of the body is given 950 r. If homologous or heterologous bone marrow cells are injected into such mice, rejection occurs and as a result of the ensuing immune interaction, death follows more rapidly than in the irradiation controls. In a series of comparison experiments we studied the effects of protecting the marrow contained within an 11-mm segment of the tail in $(C_3H \times 101)F_1$ mice irradiated with 950 r, and found that it resulted in dramatic enhancement of survival. At 950 r, 60 per cent of the mice survived at 30 days, and even at doses as high as 1150 r, 28 per cent survived. Many of these mice are still living after six months and appear healthy. When 100 million rat bone marrow cells were injected into $(C_3H \times 101)F_1$ mice irradiated with 950 r, two-thirds of the mice survived whether the tail marrow was protected or not, and alkaline phosphatase studies on the 8 out of 12 mice that survived the tail-shielding procedure showed that neither granulocytes nor erythrocytes of rat origin were present at 20 days. One must therefore conclude that the injected rat cells were rejected, since lethally-irradiated mice

with unshielded tails show the presence of rat granulocytes and erythrocytes.

To test the effect of homologous marrow on tail-shielded mice, a variety of combinations of genotypes differing at the H-2 locus, as well as combinations of identical genotypes were used. Survival was 100 per cent in pair combinations genetically identical at the H-2 locus. Unlike the effect produced by Peyer's patch shielding, no immune interaction leading to acute mortality resulted, even when homologous combinations differing at their H-2 loci were used. On the contrary, when the experimental combination $(C_3H \times 101)F_1$ mice $(H\text{-}2^k)$ was irradiated with 950 r (either with or without tail shielding) followed by ten million strain A/Tex $(H\text{-}2^a)$ bone marrow, there was enhanced survival in the group with shielded tails. In confirmatory experiments, 80 to 90 per cent survived consistently, developing no symptoms of secondary disease. Survival was also enhanced when mice with shielded tails were given x ray doses as high as 1050 r followed by homologous marrow. Sublethal doses as low as 600 r had no adverse effect on $(C_3H \times 101)F_1$ mice treated with A/Tex marrow after shielding of the tail during irradiation.

Hematologic studies on $(C_3H \times 101)F_2$ mice showed that a profound anemia and leukopenia were the immediate result of irradiation at 950 r regardless of subsequent treatment. All tail-shielded mice recovered from this anemia by 3 to 4 weeks and

remained normal thereafter. Mice given strain A/Tex marrow but no tail-shielding recovered most rapidly of all, but normal levels were maintained only until secondary disease appeared.

This experimental approach provides a tool for studying the regenerative ability of the tail marrow under stress. Under normal conditions bone marrow in the mouse tail is relatively inactive and probably contributes minimally to the steady state of the circulating hematopoietic cells. Following shielding of an 11-mm segment of the mouse tail and exposure of the body to 950 r, hematopoiesis is greatly intensified in the shielded segment. Thus it is obvious from these experiments that a relatively inactive segment of bone marrow can be made to proliferate under stress in situations such as total-body x irradiation. It is equally obvious that the hematopoietic cells in an 11-mm segment of the tail (estimated to be fewer than 700,000) are capable of effectively repopulating the bone marrow and spleen so that survival from otherwise lethal dosages of x irradiation is greatly enhanced.

Following exposure to 950 r and whole-body x irradiation with 11 mm of the tail shielded, colonization of the spleen is microscopically apparent at 4 days and macroscopically apparent by 6 days as the clones increase in size.

It is interesting but not surprising that complete recovery of the blood-forming tissue, as judged by histologic study or the

hematological studies of the peripheral blood, is less rapid than in spleen-shielded animals exposed to 950 r whole-body x irradiation, or than in mice exposed to 950 r x irradiation and then given 10^6 isologous bone marrow cells. The shielded segment of the tail provides a smaller number of stem cells for repopulation or proliferation than the shielded spleen or 10^6 injected isologous bone marrow cells.

No attempts have yet been made to ascertain whether the cell population in the lymph nodes or femoral marrow of surviving mice which received tail-shielding followed by homologous marrow is of host or graft origin. However, in view of the absence of secondary disease, the assumption that the donor cells have been rejected and that final repopulation has been accomplished by the shielded tail segment, seems plausible.

Dr. Charles Huggins made the observation that the tail of the rat, which normally has an inactive fatty marrow, will become an active hematopoietic tissue when inserted into the body cavity. This transformation of a fatty yellow marrow to a red marrow was considered to be related to the increased temperature within the body cavity as compared to the normal condition.

In view of the fact that the hematopoietic tissue of a shielded 11-mm segment of the tail of a mouse exposed to a lethal dose of x irradiation was greatly intensified, we studied this same phenomenon in rats. Adult Sprague-Dawley rats normally have

a fatty marrow in the tail, conservatively estimated as 99 per cent fat and 1 per cent cells. Hematopoietic cells, though sparse, are present and all cell types are represented. Shielding of the entire tail of the rat during exposure of the body to 750 r x irradiation resulted in 33 per cent survival.

Histologically no intensification of hematopoiesis is apparent in the shielded rat tail marrow.

Since after tail shielding 33 per cent survive an otherwise lethal dose of x irradiation one must assume that effective repopulation occurred in sites peripheral to the tail.

Control of Infection in Radiation Death

E. L. Simmons, C. Pierce and N. Larkin

In recent years radiation research in many laboratories has been hampered because of a high incidence of Pseudomonas aeruginosa in mice. Such mice appear to be healthy in the stock colony, but even low doses of radiation will weaken their immune defenses, a massive overwhelming invasion follows, and death ensues as early as the fourth or fifth day. In experiments involving supra-lethal radiation doses, it is impossible to save such infected mice with spleen shielding, injection of bone marrow cells, or by chemical protection.

The mistake is often made of testing for this infection by fecal cultures. Because Pseudomonas more often appears as an upper-respiratory infection, it is transmitted by mouth to the

water bottle, and is spread thence to other animals in the cage. It is very simple to screen colonies of mice by sampling the water bottles and culturing these samples in glycerol broth. Furthermore, it is possible to prevent Pseudomonas growth in the bottle (and hence its spread) by acidifying the water to pH 2 by HCl, or by chlorinating it at 20 PPM. Chlorinated bottles must be changed every other day, but acidified water prevents the growth of Pseudomonas for over a week. No antibiotic that we have tried will cure the mice, but survival after irradiation is best when the animals are given streptomycin sulfate in their water for at least 2 weeks after irradiation.

Another organism that has been implicated in early radiation death is Citrobacter freundii, the presence of which can be tested for by making smears of the colon lining on MacConkey's medium. Fortunately this organism can be controlled by antibiotics, and we have found both streptomycin sulfate and neomycin to be extremely effective.

STUDIES WITH HIGH ENERGY RADIATIONS

High Energy Radiation Sources, Their Development and Maintenance

L. S. Skaggs

High Energy Radiation Therapy Sources. Three high energy radiation sources were installed in the Argonne Cancer Research Hospital for research in radiation therapy. These, in the order in which they became available for therapy, are the 2-MeV Van de Graaff x ray generator, the rotational cobalt-60 therapy machine, and the 50-MeV electron linear accelerator.

Van de Graaff X ray Generator. The Van de Graaff is a commercial machine and became operational in 1953. A few minor improvements have been made, including a correction for output variation with angle of inclination of the generator. An extensive set of isodose curves in water was developed.

Cobalt-60 Therapy Machine. The cobalt-60 therapy machine is an original design, built in the Argonne Cancer Research Hospital shop, incorporating several unique features. The cobalt source is 3/4 cm diameter by 3 cm long and features the smallest effective source size of any kilocurie unit yet available. A new source, soon to be installed, will have an activity of 4000 curies, or 350 curies per gram, and will be, as far as we can learn, the highest specific activity kilocurie source in use.

The shield is of uranium, resulting in considerable reduction in size and a factor of 4 in weight over the conventional lead. The shutter mechanism is positive and reliable and does not involve movement of the source. The source reloading scheme is fast and safe and does not disturb adjustment of the machine.

Linear Accelerator. The linear accelerator was built by commercial contract following the design of the Stanford accelerator. During construction and for a short time after installation of the accelerator proper, a study was made of various schemes for electron beam application. The final design of an offset and 90° deflected beam with a scanning mechanism for covering the tumor area was chosen and a commercial contract negotiated for detailed engineering design and construction. Rather extensive improvements to the accelerator were undertaken to increase beam stability and reliability of the machine. After installation of the beam deflecting system, some mechanical modifications were necessary to increase the rigidity of the third magnet and to maintain proper electron beam position for all orientations of the deflecting system. The machine became operational for patient therapy in 1959. A number of dose distribution studies have been made and a careful determination of absolute dose in rad units was made. A program is being developed to calculate the dose at various depths for any size or shape of field and is being undertaken using digital computer methods. A new high current electron

gun is also being installed in the linear accelerator. This will permit additional use of the accelerator for production of short-lived isotopes, such as oxygen-15, for diagnostic purposes.

The Analog Computer

L. S. Skaggs

An analog computer, purchased and supported largely by Public Health Service funds, is installed in the Argonne Cancer Research Hospital and has been used on several Argonne Cancer Research Hospital projects. The computer is a general purpose iterative and real-time Beckman instrument incorporating 68 operational amplifiers usable as integrators (36), summers and inverters. It also has 28 multipliers (6 programmable as dividers), 3 sine-cosine generators, 8 arbitrary function generators, 110 coefficient potentiometers, a noise generator and an 8-channel display oscilloscope. Terms of the grant provide computer service for all departments of the Division of Biological Sciences.

Problems of interest to the Argonne Hospital that have been done or are under investigation include the following:

1. Determination of useful compartment model for respiratory gases (Dr. Paul V. Harper, Jr.). Dr. Harper is trying to construct a virtual compartment model for respiratory gases by fitting the solutions of simultaneous differential equations

describing the model to his experimental curves. Difficulties have been encountered, but a new approach using a method of Fourier transformers is being tried.

2. Solution of Modified Lamm Equation. Solution of the partial differential equation describing the molecular weight distribution in the ultracentrifuge as a function of radial distance and time was performed by a separation of variables and solution of the eigenvalue problem. This provided solutions satisfactory for large values of time. A solution by finite differences appeared to be better for the values of time encountered in the experiments and is now being investigated for large values of molecular weight.

3. Calculation of radiation therapy dose. A bivariant function generator gives the value of dose in a beam of radiation as a function of depth within the tissue and position across the width of the field. Distributions for any size of field can be generated from sets of isodose curves determined from just 3 field sizes of the particular quality of radiation to be used. The computer enables the resulting dose in the patient to be summed and determined for as many as 4 fixed fields. A method is under development to extend this to rotational therapy.

The Therapeutic Application of High Energy Sources

J. W. J. Carpender

Intercomparison of orthovoltage and supervoltage radiation in the management of malignant disease has continued since the opening of the Argonne Cancer Research Hospital. The devices available are the 50-MeV Linear Electron Accelerator, the 2-MeV Van de Graaff Generator and the large cobalt-60 rotator. Recently a small 30-curie cobalt-60 unit has been added to the two orthovoltage machines in the Chicago Tumor Institute. Enough patients have not yet been accumulated in the various categories to assess results.

A cooperative study with the Ear, Nose and Throat Service of Billings Hospital, has been set up where certain tumors are randomly selected for treatment with pre- or post-surgical radiation. Tumors such as those of the hypopharynx have a very poor prognosis with any method of treatment and it is hoped that one or the other of the combined treatment plans will show increased survival.

Preoperative radiation for carcinoma of the oesophagus is being studied with a hope of increasing the survival rate in this condition.

In cooperation with the Department of Surgery, we are participating in the national evaluation of the management of lung malignancy.

Patients having inoperable or recurrent carcinoma of the stomach or large bowel have been treated with radiation combined with intravenous colchicine. This seems to hold some promise of better palliation than radiation alone offers. In the same line, actinomycin D has been used in some of the radioresistant sarcomas.

Within the last few months, alteration in the time dose schedule used in treating painful bony metastases has been made in an effort to find a schedule which will more quickly give relief of pain.

Effects on Mouse Hair Roots Produced by X Ray Irradiation Combined with Radiopotentiating or Radioprotective Compounds

F. D. Malkinson and M. L. Griem

A method of microscopic examination of plucked mouse anagen hairs has been used to study the radiosensitizing effects of colchicine, actinomycin D, and triiodothyronine. For colchicine and actinomycin D quantitative studies have demonstrated a distinct synergistic effect on hair damage produced by irradiation. Critical factors are the administration sequence of drug-radiation and the time periods separating drug injection and x-ray exposure (16 hours for colchicine, 4 and 16 hours for actinomycin D). The significant increase in hair matrix damage induced by the

combination of these agents has led to current evaluation of their usefulness for treating malignant lesions in experimental animals and in humans.

Triiodothyronine was found to enhance radiation damage uniformly at varying x ray dosage levels, while hypothyroid animals showed less radiation effects than control animals.

The same mouse hair indicator system has been employed for a quantitative investigation of the protective effects of 2-mercaptoethylamine on x ray induced dysplasia. Studies in a dosage range of 400 to 1000 r revealed a dose reduction factor of 1.5, with less protection afforded at the high dosage levels. Enhanced damage to hair matrices was found when administration of 2-mercaptoethylamine immediately followed, rather than preceded, radiation.

The technique offers a rapid, quantitative, reproducible and non-destructive method for evaluating agents or factors which predispose to, induce, or protect against cellular damage. Currently a method is being devised to measure directly the incorporation of isotope-labeled amino acids into anagen hairs so that damaging effects of radiation and chemotherapeutic agents can be detected by a reduction in radioactivity of examined hairs.

Modification of Radiation Response of Tissue by Actinomycin -- Preliminary Clinical Evaluation

M. L. Griem and K. Ranniger

Preliminary experimental studies on the effects of combining parenteral actinomycin D and irradiation were conducted on mice, using a microscopic hair indicator system to evaluate the extent of cellular damage to the anagen hair matrix induced by the combined use of actinomycin D and irradiation. These experimental studies showed that an interval of 3 to 4 hours between drug administration and irradiation produced the maximum degree of dysplasia in the hair. Similar experiments were conducted using solid transplantable Walker 256 rat tumors, and solid transplantable myeloma tumor in the C3H mouse. Again the phases of increased radiosensitivity and radioresistance were distinguishable as the parameters of time interval was varied between injection of drug and radiation. Subsequently, patients with advanced malignancies were studied employing usual palliative doses of radiation therapy up to a total of between 2000 and 3000 rads. Patients were given intravenous injections of 0.5 mg of actinomycin D followed after 3 to 4 hours by a 500-rad minimum dose delivered to the tumor. This sequence was repeated twice weekly for 2 to 3 weeks. Patients with a number of unusual tumors, usually considered radioresistant, were treated.

Modification of Radiation Response of Tissue by Colchicine -- Clinical Evaluation of Tumor Response

M. L. Griem and F. D. Malkinson

Before clinical trials were initiated an experimental study was carried out to evaluate the combined effects of irradiation and intravenous colchicine on animal tumors, and on anagen hair in the mouse. These experiments showed that an interval of 16 hours between drug administration and irradiation produced the maximum response in tumors and the maximum injury to the hair. Subsequently, the parameter of time interval between parenteral administration of colchicine and radiation was evaluated in patients with the cutaneous tumor mycosis fungoides, and here again, a 16-hour interval produced maximum response. Patients with advanced malignancies were then studied employing usual palliative doses of radiation therapy up to a total of 4,000 rads. Subjects were given intravenous injections of 4 mg to 5 mg of colchicine and 16 hours later a 500 rad minimum dose was delivered to the tumor. This sequence was repeated every 5 days for 8 treatments.

Over 100 patients with advanced malignancies have been treated. The most favorable response was obtained in cases of adenocarcinoma of the stomach, colon, pancreas and lung. Excellent response has also been seen in patients with mycosis fungoides and certain squamous cell carcinomas of the skin. No significant

response was obtained in patients with renal cell carcinoma of the kidney. Similar results have been obtained by Bonomini and Fiorentino, and confirm our observations.

Objective response has been demonstrated by long-term survival without recurrence of the primary lesion or metastases, or by dramatic shrinkage of the tumor in comparison to areas treated with colchicine alone or radiation alone in the same fractionation and sequence. Toxic manifestations of colchicine consisting of diarrhea and transitory leukopenia have been seen in several cases. Hair loss has not been observed.

Studies by Walaszek and co-workers on the parenteral distribution of radioactive colchicine show that this drug is retained preferentially in tumors and in the gastrointestinal tract. This may explain the observed response of adenocarcinoma of the gastrointestinal region.

Chemical Modification of Radiation Effect in Mice

E. L. Simmons

It is well established that mice can be protected against x rays or gamma rays by pretreatment with S,2-aminoethylisothiuronium (AET) or with p-aminopropiophenone (PAPP), but the comparative protection against high energy electrons afforded by such substances has never been explored. In addition to its basic radiobiologic importance, such a study is of increasing interest

in space medicine because of the possible exposure of astronauts to radiation from the Van Allen Belts. In our studies, we have compared the effects of 30 MeV electrons from the Argonne Cancer Research Hospital Linear Accelerator with conventional 250 kvp x rays from a G. E. Maxitron.

Mice were confined in lusteroid tubes suspended in a water phantom, and were irradiated either awake or under Nembutal anesthesia. Nembutal exerted no effect on survival after irradiation with electrons, but was slightly protective against x rays. Our experiments indicated that pre-irradiation treatments with AET or with PAPP were able to reduce 30-day lethality in mice exposed to high energy electrons. AET afforded better protection than did PAPP against electrons as well as against x rays. The 30-day LD_{50} value for mice exposed to 30 MeV electrons was 954 ± 20 rads; pre-treatment with PAPP increased the LD_{50} to 1323 ± 28 rads, and with AET to 1528 ± 85 rads. The 30-day LD_{50} value for anesthetized x-rayed mice was 826 ± 50 rads; pre-injection of PAPP increased this to 985 ± 25 rads, and of AET to 1027 ± 36 rads.

Earlier experiments showed that pre-treatment of mice with estradiol benzoate 10 days before x irradiation resulted in increased survival. We have now tested estradiol, estrone, and estriol and have obtained protection with all three. The doses required, however, were 5 times greater than was needed with

the benzoate ester. Unlike other classes of chemical protectors such as AET and PAPP, which must be given shortly before irradiation, maximum survival with all the steroids tested occurred when they were given 10 days before, and not immediately before the time of irradiation, when they would be present in the body in greatest concentrations. Thus, in some fashion the estrogens trigger a biological reaction that has its maximum effect 10 days later, and we are re-examining this interesting phenomenon. Peripheral blood counts made 10 days after estradiol and estriol administration did not show any changes from initial control levels. Following 500 r, however, although white cell counts diminished precipitously, they recovered more rapidly in the groups treated with estrogen, while platelets and hematocrit values did not fall as markedly and recovered earlier. It has not been determined whether estrogens prevent damage to stem-cell precursors, or stimulate mitosis in hardy cells that were not killed by the irradiation.

Finally, in experiments to test the protective action of dimethyl sulfoxide (DMSO), the LD_{50} for CF No. 1 female mice exposed to x rays was increased from the control value of 630 r to 780 r when they were treated with DMSO. Combined pre-treatment with estrogen and DMSO, and also with DMSO and AET, proved to be more effective than any of these substances alone.

Physical and Biological Investigations with High Energy Radiations

L. H. Lanzl

Rather early in the history of the Argonne Cancer Research Hospital, a decision was made to include in the hospital high energy radiation equipment, principally for cancer therapy. As a result of this decision, a 2-MeV Van de Graaff generator was purchased and installed, a moving-field cobalt-60 therapy unit was designed and manufactured at the Argonne Hospital, and a unique 5 to 50 MeV travelling wave electron linear accelerator, together with a beam deflecting and scanning unit, were specified and acquired through a series of contracts. The linear accelerator program presented the greatest challenge because very many new concepts were employed.

Following the installation and operation of the above equipment, a separate project was formed to carry out a program of physical and biological research emphasizing the utilization of the high-energy equipment for radiation therapy.

Patient Treatment Planning. Among the initial steps in using a new machine for radiation therapy is the determination of its output under various conditions of operation, together with isodose distributions and animal irradiation checks. The purpose of our present program is to improve radiation treatment by increasing the accuracy, speed and automation of treatment planning.

To this end, several new instruments and techniques have been designed and are being put into service. To see how they are utilized, consider the steps that might be taken in planning the treatment for a typical patient for rotation therapy.

First an Automatic Patient Contour Plotting Device is used for rapid and accurate measurement of the patient's contour (see abstract). Then a field size, center of rotation, and limits of the sector of rotation, if any, are chosen. This information is utilized in the Analog Computer Calculation of Rotation Isodose Distributions (see abstract).

If experimental verification of the calculation is required, measurements of the distribution can be made by film, ion chambers, or thermoluminescent dosimeters in a heterogeneous phantom. To obtain the dose distribution quickly in those cases where film dosimetry is employed, A Semiautomatic Isodose Curve Plotter has been designed and put into service. This unit has built-in electronic dose linearizing circuits to simplify analysis of optical density measurements (see abstract). As a further aid in checking the accuracy of the calculated and measured distributions the transmission dose is detected by an ion chamber mounted on the cobalt-60 therapy unit.

Although the Van de Graaff generator can be used for rotation therapy, the cobalt-60 unit has been found most suitable for this form of treatment. One of the more unusual features of

this particular unit is the high specific activity, over 300 curies per gram, of the source. This permits high total activity in a small size and reduces treatment time without sacrificing sharp beam definition.

Scanning Electron Beam. The uniqueness of the electron beam deflection and scanning device which operates in conjunction with the linear accelerator requires techniques of treatment planning and dose calculation quite different from those used in fixed field or rotation with gamma- or x-ray beams.

This has led to a number of studies requiring the use of digital computers:

1) A fundamental study of the deposition of energy from high energy electrons in water-like media. The necessary calculations start from the basic physical formulations of collision and radiation loss by electrons and of multiple scattering of electrons.

2) A calculation of the saturation of an electron beam monitor chamber under pulsed and scanning conditions.

3) A digital computer calculation of dose distributions for patient treatment, including electron energy, depth, field sizes and shapes. The distribution in a plane at the depth under consideration is determined for the 5-mm diameter elemental beam, using photographic film. Numerical values of the dose at one-half cm intervals in each direction within the plane are fed to the

computer. A point is now chosen, either within or outside the irradiated field at which it is desired to know the dose, and the computer is programmed to sum all the contributions from the elemental beam as its motion is simulated in one-half cm steps over the entire field (with L. S. Skaggs).

Radiation Hazard. Awareness of the potential danger of the hazardous amounts of radiation used has prompted two projects in health physics during this past year:

1) A multidisciplinary symposium was held in Chicago, the proceedings of which are now being published under the title RADIATION ACCIDENTS AND EMERGENCIES IN MEDICINE, RESEARCH, AND INDUSTRY (edited by L. H. Lanzl, J. H. Pingel, Argonne National Laboratory, and J. H. Rust, Department of Pharmacology, The University of Chicago).

2) The use of radium in a department carries with it the possibility of radium contamination should a tube or needle rupture. A Radium Leak Detector has been designed and is being built for the daily monitoring of all the radium in storage (see abstract).

Bone Mineral Study. Other work has also been undertaken utilizing certain radioactive materials. The low energy gamma radiation is used in the Measurement of Bone Mineral Using a Radioisotopic Device consisting of a small source of iodine-125 and a scintillation detector (see abstract). Those measurements are useful in detecting bone mineral changes and following the course of any prescribed therapy.

Automatic Patient Contour Plotting Device

L. H. Lanzl, L. Bess and M. L. Rozenfeld

During radiation therapy planning, a contour of the patient at the tumor level is often needed. The existing methods for obtaining such contours are somewhat tedious and time-consuming. Therefore, a device has been constructed which is capable of carrying out this function accurately and quickly.

The device has been mounted on the Argonne Hospital's cobalt-60 therapy machine in such a way that it rotates about the patient. The device detects the distance from its surface to that of the patient. The contour is then produced by subtracting this distance from the constant distance to the center of rotation over the entire circular path.

The distance measurement is performed by means of an optical-electronic system composed of two slightly convergent light beams directed toward the subject. The separation of the two beam images on the patient surface thus depends on its distance from the device. A lens system focuses light images reflected from the surface on a shutter wheel, which rotates at a constant angular velocity, so that the time interval between the passage of the two light beams through a given shutter slit is a measure of the distance to the surface. The light pulses are converted into electrical impulses whose time separation is made proportional to the distance by an optical-geometrical compensating feature. This

time separation is converted to a direct-current signal fed to the radial coordinate of a recorder. The angular coordinate is determined by the angular location of the contour device.

The unit has been tested successfully on mechanical objects.

Analog Computer Calculation of Rotation Isodose Distribution

M. L. Rozenfeld

One method commonly used to calculate the dose distribution due to rotation therapy utilizes the central axis depth dose corrected for inverse square law only. These central axis data are used to determine the dose at a point of interest in the patient as the beam revolves around the center in increments of 15°. The dose at this point, due to the rotation, is then the average of doses from each angle of entrance. This process is repeated for new points until sufficient data are available to interpolate the resultant isodose curves.

An analog computer program has been developed to automate this process. Most of the approximations made in the procedure outlined above are still required, except that continuous integration is performed instead of summing the results at 15° steps. Since the computer will integrate the dose at any point, due to a complete rotation, in less than a second, it is possible to program a feedback system to search out the isodose lines. Thus the

output of the program is the complete set of isodose curves instead of a table of values from which the curves will have to be drawn by interpolation.

A Semi-Automatic Isodose Curve Plotter

M. L. Rozenfeld, H. Vetter and L. H. Lanzl

A device for plotting isodose curves from film has been constructed and used for several months. A circuit is incorporated which corrects for any nonlinearities in both the densitometer and the film, so that the final results represent the true isodose and not merely isodensity curves.

The plotter utilizes twelve independent gate circuits, each one opening when the linearized densitometer output is equal to a different reference level. Pulses with a 2 kilocycle repetition rate pass through any of the gate circuits which are open, and write on the spark-sensitive paper. Optimization of writing versus searching time is achieved by the use of manual scanning in full view of the spark-sensitive paper. The results for a single film are obtained in 10 or 15 minutes with an accuracy of ± 2 per cent.

The device was constructed for measuring and plotting isodose contour lines from films embedded within a phantom exposed to external, therapeutic beams of radiation, but was also found useful in a scanning system for patients with internal radioactive depositions where film is used in the final output stage.

Radium Leak Detector

M. L. Rozenfeld and E. W. Mason

A system for the daily checking of leaks in the entire stock of radium contained in a safe is being developed. This is to be accomplished by monitoring the air in a sealed housing containing the radium safe. The air in the housing will be recirculated, by a pump, through a membrane filter which is facing a solid state alpha detector. The detector is connected to a log count rate meter, alarm circuits, and strip chart recorder.

An alpha detector was chosen for this purpose for several reasons. Suitable discriminator circuits can make the detector insensitive to gamma rays, thereby allowing it to be situated near the safe without affecting the background alpha count. The background count is further reduced by recirculating the air in the sealed housing and thereby eliminating the natural radon in room air.

The pump will be operated for 1 hour a day which is about 3 times the half-life of the radon daughters. The detector circuit will be turned on with the pump but will be operated for a period of 2 hours. The 1-hour pumping time is sufficient for the activity of the radon daughters collected on the filter to approach within about 85 per cent of equilibrium with the activity of the radon gas in the housing. The additional hour of detection after the pump is shut off will show a decay with a 20-minute half-life in the event of a radon leak, or no decay in the event of a radium leak.

Among the advantages of this system are its high sensitivity, its ability to distinguish between radon and radium leaks, the complete elimination of personnel dose involved in the testing procedure, and the assurance of prompt detection after a leak develops.

Measurement of Bone Mineral Content Using a Radioisotopic Device

L. H. Lanzl and N. M. Strandjord

An accurate measurement of the mineral content of bone *in vivo* has long been a medical problem. An instrument using radioactive iodine-125 has been devised for non-destructive testing to determine mineral changes in the skeleton. This is accomplished by measuring the transmission of a small beam of radiation emanating from iodine-125 through a single human finger bone. (For small animal work, the rear leg is used.)

Radiographs must be used for accurate positioning of the organ under consideration. Built into the unit is an automatic repositioning feature coupled with an automatic print-out device. An error analysis indicates that the absorption coefficients of bone *in vivo* can be determined to an accuracy of ± 1 per cent.

With Drs. Edward Davis and Edward Person, we have undertaken the study of possible beneficial effects of estrogen therapy in the postmenopausal female. We are measuring the mineral content of a group of patients who have had bilateral oophorectomy and

who are endogenously estrogen deficient. Those patients who have received exogenous hormone therapy in the postoperative period are being compared with postmenopausal females who have not had their ovaries removed and are not on hormone therapy. Measurements on the bones of females in the premenopausal era serve as controls. The results thus far, which are based on one hundred patients, show that the group receiving hormone therapy appears to have a linear absorption coefficient halfway between the other two, indicating a beneficial effect on the bone mineral content.

In another study which we are undertaking with Drs. Paul V. Harper and Edward Paloyan, we are measuring the linear absorption coefficient in the leg of a rabbit on an enriched cholesterol diet. In a first run of three test and three control rabbits, one of the test rabbits exhibited a rising cortical bone coefficient, although the bone had not increased in size more rapidly than those of the control rabbits.

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