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DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH

ANNUAL TECHNICAL REPORT 1981

Eliezer Huberman, Director
Thomas E. Fritz, Associate Director
Marcia W. Rosenthal, Editor

June 1982

Preceding Report
ANL-81-50, August 1981

ABSTRACT

This report summarizes research during 1981 in the Division of Biological and Medical Research, Argonne National Laboratory. Studies in Low Level Radiation include comparison of lifetime effects in mice of low level neutron and gamma irradiation, delineation of the responses of dogs to continuous low level gamma irradiation, elucidation of mechanisms of radiation damage and repair in mammalian cells, and study of the genetic effects of high LET radiations. Carcinogenesis research addresses mechanisms of tumor initiation and promotion in rat liver, chemical carcinogenesis in cultured mammalian cells, and molecular and genetic mechanisms of chemical and ultraviolet mutagenesis in bacteria. Research in Toxicology uses a variety of cellular, whole animal, and chronobiological end points, chemical separations, and statistical models to evaluate the hazards and mechanisms of actions of metals, coal gasification by-products, and other energy-related pollutants. Human Protein Index studies develop two-dimensional electrophoresis systems for diagnosis and detection of cancer and other disease. Biophysics research includes fundamental structural and biophysical investigations of immunoglobulins and key biological molecules using NMR, crystallographic, and X-ray and neutron small-angle scattering techniques. The final sections cover support facilities, educational activities, seminars, staff talks, staff, and funding agencies.

TABLE OF CONTENTS

1. Division Director's Introduction
E. Huberman.....1

LOW LEVEL RADIATION

2. Neutron and Gamma Ray Toxicity Studies
J. F. Thomson, D. Grahn, L. S. Lombard,
F. S. Williamson, K. H. Allen, G. L. Holmblad,
J. L. Hulesch, V. A. Ludeman, A. R. Sallese,
E. F. Staffeldt, J. E. Trier, and B. J. Wright.....7

3. Radiation Toxicity in Dogs
T. E. Fritz, L. S. Lombard, C. M. Poole
T. M. Seed, D. V. Tolle, J. M. Angerman,
S. M. Cullen, D. Doyle, L. V. Kaspar,
W. G. Keenan, A. R. Sallese, K. Jacaruso,
and L. Lanning.....11

4. Radiation-Induced Hemopathologies
T. M. Seed and L. V. Kaspar.....15

5. Radiation Biology of Cultured Mammalian Cells
A. Han, M. M. Elkind, E. Ben-Hur, F. Buonaguro,
C. K. Hill, H. Utsumi, F. Suzuki, E. M. Buess,
J. L. Dainko, P. J. Dale, and C. M. Liu.....19

6. Mammalian Genetics
D. Grahn, M. L. Garriott, B. H. Farrington,
C. H. Lee, J. J. Russell, and D. J. Crowe.....23

CARCINOGENESIS

7. Mechanisms of Hepatocarcinogenesis
C. Peraino, J. E. Morris, W. E. Boernke,
V. A. Ludemar, A. M. Prapuolenis, and
E. F. Staffeldt.....29

8. Mutagenesis and Cell Differentiation in Chemical Carcinogenesis
E. Huberman, C. A. Jones, E. A. Malvoisin,
S.-I. Murao, B. Ryffel, I. Simon,
M. F. Callaham, C. B. Henning, and
B. A. Sedita.....33

9. Molecular and Genetic Mechanisms of Mutagenesis
H. E. Kubitschek, P. R. Reynolds, and
D. Williams-Hill.....37

10. Molecular and Cellular Effects of Solar Ultraviolet Light
M. J. Peak, J. G. Peak, R. W. Tuveson,
M. A. Turner, and L. A. Nerad.....41

11. Mechanisms of Damage and Repair by UVA, UVB, and UVC
R. B. Webb.....45

TOXICOLOGY

12. Metal Metabolism and Toxicity
M. H. Bhattacharyya, R. P. Larsen, P. A. Benioff
H. C. Furr, E. S. Moretti, R. D. Oldham,
D. P. Peterson, M. I. Spaletto, and
M. E. Shackelford.....51

13. Metal Toxicants: Carcinogenesis and Mitigation
Y. E. Rahman, P. A. Lagocki, R. A. Schwendener,
E. A. Cerny, K. Rogers, and J. J. Terfruchte.....55

14. Toxicology of Coal Gasification
C. A. Reilly, Jr., F. R. Kirchner, T. Matsushita,
M. J. Peak, K. E. Wilzbach, R. E. Jones,
J. M. Leonardo, G. Kaufman, K. T. Blankenship,
R. M. Bressette, M. Cannella, B. Crespi, and
M. A. Nocera.....59

15. Toxicology of Coal Gasification: Chemical Characterization
D. A. Haugen, M. J. Peak, V. C. Stamoudis,
A. S. Boparai, C. A. Reilly, Jr.,
K. E. Wilzbach, K. M. Suhrbier, S. S. Dornfeld,
D. Venters, and F. J. Tremmel.....65

16. Neurobehavioral Chronobiology and Toxicology
C. F. Ehret, A. L. Cahill, R. S. Rosenberg,
P. H. Duffy, K. R. Groh, J. J. Russell,
J. V. Barnett, A. S. Gulczynski, R. Scott,
and G. H. Thomsen.....69

17. Biostatistics and Health Impacts of Energy Technologies
M. E. Ginevan, B. A. Carnes, J. J. Collins,
C. D. Brown, J. R. B. Curtiss, N. Devine,
and A. Rock.....73

THE HUMAN PROTEIN INDEX

18. The Human Protein Index

N. G. Anderson, N. L. Anderson, J. J. Edwards, C. S. Giometti, J. Taylor, K. E. Willard, M. A. Gemmell, S. L. Nance, A. E. Scandora, S. L. Tollaksen, B. Mallard, and M. T. Powers.....79

BIOPHYSICS

19. Properties of Biological Molecules in Solution
M. MacCoss, H. M. Schwartz, C. F. Ainsworth, S. H. Gray, M. McCroskey, and R. J. Ross.....87

20. Structural Studies of Immunoglobulins
M. Schiffer, M. T. Short, F. J. Stevens, and F. A. Westholm.....91

21. Neutron and X-Ray Small-Angle Scattering
C. S. Borso, B. I. Berkoff, and C. T. Mizumoto.....95

APPENDICES

22. Support Facilities.....101

23. Educational Activities.....105

24. Divisional Seminars, January 1981 through June 1982.....111

25. Outside Talks by Divisional Staff during 1981.....117

26. Publications Appearing in Calendar Year 1981.....129

27. Staff and Funding Agencies.....143

AUTHOR INDEX.....151

1. DIVISION DIRECTOR'S INTRODUCTION

The directorship of the Division of Biological and Medical Research changed in August of 1981 when I became the Division Director. I want to thank my predecessor, Dr. Douglas Grahn, for his assistance in a smooth transition, and to wish him well in his desired and much deserved return to full time research.

The major portion of this report consists of 20 brief project summaries. They are primarily concerned with the progress of research during the calendar year 1981 and the early part of 1982, but to provide better continuity and perspective they cover the principal activities of each project over a period of approximately two years. They have purposely been kept short, so they do not necessarily summarize all the work that has been done. Following each project report are listed the publications pertinent to the project that are dated 1981 and 1982. The list includes publications that were in press (accepted for publication) as of May 1, 1982.

The project summaries are followed by sections indicating the range of other activities in the Division. They describe the animal and the radiation facilities, the varied graduate and undergraduate level educational activities, the Divisional seminars presented by visiting and in house speakers, the principal outside talks given by the staff during 1981, and an inclusive list of Divisional publications - journal papers, reports, and abstracts - that appeared during the calendar year 1981. The last section of this report presents the staff and the Governmental agencies supporting each of the projects.

For the purposes of this report, we divide the Division into five program areas which cut across the divisional group structure, illustrated in the following figure, in order to emphasize broad scientific relationships. These areas are:

Low Level Radiation

Carcinogenesis

Toxicology

The Human Protein Index

Biophysics

Research in biological effects of Low Level Radiation deals with the critical issues concerning the safety of nuclear technology, namely the assessment of health effects of exposure of man to ionizing radiation. Ongoing studies, presented in the first five of the following project summaries, include research programs that deal with effects at three different levels of biological organization, somatic effects in whole animals, genetic effects in whole animals, and effects in cultured mammalian cells.

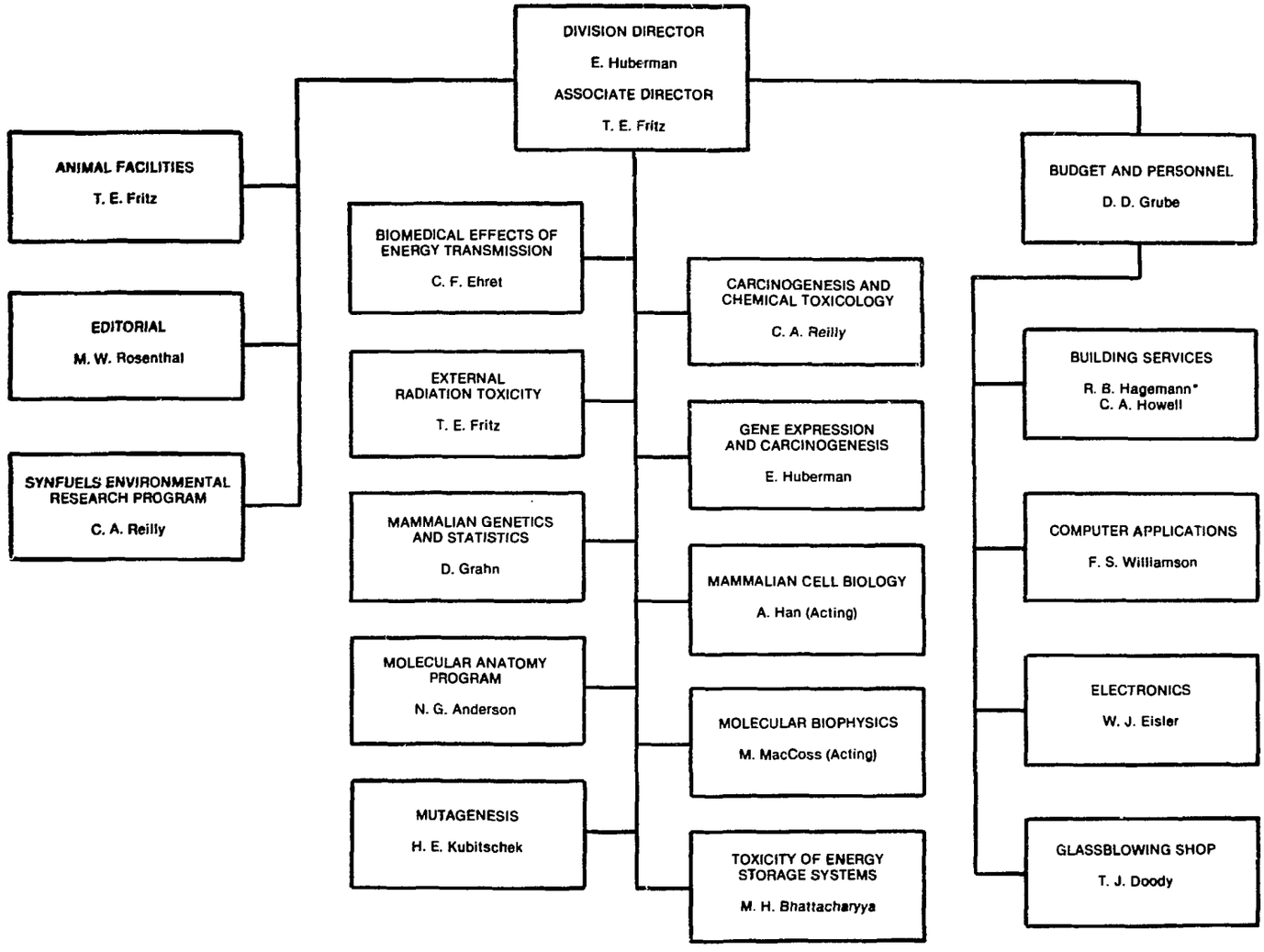
The multifaceted Carcinogenesis program area brings together five fundamental mechanistic and developmental research studies. Chemical carcinogenesis is the primary focus of a project dealing with initiation and promotion in rat liver and in another project concerned with mutation and differentiation in cultured mammalian cells. Nonionizing radiations, both broad spectrum and monochromatic, are the principal inducing agents in three projects using a variety of bacterial strains as well as mammalian cells.

Research in Toxicology is reported in six project summaries. The first two identify and measure the toxicity and carcinogenic potential of metals, particularly those related to the production and storage of energy, and include studies directed toward mechanisms and mitigation of toxicity. The second two are concerned with the toxicology of process streams of synfuel technologies, particularly coal gasification, using a multitiered biological approach closely coordinated with chemical characterization of the process stream components. The fifth project deals with the measurement of neurobehavioral responses, focusing on changes in established biological rhythms, while the sixth develops statistical methodology relevant to the assessment of health impacts of energy technologies.

The research related to the Human Protein Index in a sense contains elements of all three of the preceding program areas. It has developed two-dimensional electrophoresis and computerized data reduction methods as the basic tools to establish an annotated index of human proteins. The index will include data on normal proteins, genetic variants, and disease-related changes.

The principal goal of the three projects in Biophysics is the development of fundamental structure and conformation information for biological molecules at all levels of organization. This information is basic to our ultimate understanding of biological events and accurate predictions of toxicologic insults. The first project is concerned with chemical and structural properties of biological molecules in solution, the second with crystallographic and chemical studies of immunoglobulin structure, and the third with the development and use of neutron and X-ray small-angle scattering to study the structure and dynamics of individual biological molecules and of complexes of interacting molecules.

E. Huberman, Director
Division of Biological
and Medical Research



3/82

DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH

*Consulting Engineer

LOW LEVEL RADIATION

2. NEUTRON AND GAMMA RAY TOXICITY STUDIES

J. F. Thomson, D. Grahn, L. S. Lombard, F. S. Williamson, K. H. Allen, G. L. Holmblad, J. L. Hulesch, V. A. Ludeman, A. R. Sallese, E. F. Staffeldt, J. E. Trier, and B. J. Wright

The purpose of the program is to obtain information on the late effects of low doses of both high-LET and low-LET ionizing radiation in experimental animals that can provide quidance for the prediction of radiation hazards to man. This information takes the form of dose-response curves for life shortening from all causes of death and from numerous specific causes. This program is closely allied with that in Mammalian Genetics (Section 6), in which genetic end points are examined using the same radiation sources, dose rates, total doses, and strain of mouse.

The experiments employ approximately 0.85-MeV fission neutrons from the JANUS reactor and ^{60}Co gamma rays, delivered as single, fractionated, or weekly duration-of-life exposures covering the following range of doses and dose rates:

	Neutrons	Gamma Rays
Single exposures (rads)	1-240	22.5-788
Fractionated, 2-60 fractions (rads, total dose)	2-230	100-3820
Duration of life (rads/weekly fraction)	0.67-2.67	7-32

In most experiments we have employed the house mouse, Mus musculus, specifically the C57BL/6 x BALB/c F_1 hybrid ($B6CF_1$). To provide an interspecies comparison, we have used the wild-type white footed mouse, Peromyscus leucopus, a cricetid rodent the same size as Mus but with a significantly longer life-span and a distinctly different spectrum of tumors.

Animals are observed for their lifetimes; gross and microscopic examinations of the decedents are carried out to establish the probable cause of death. The biological studies are supported and complemented by sophisticated dosimetric and data processing programs.

A brief summary of the current status of the results is as follows:

1) The dose-response curves for life shortening after gamma irradiation are statistically indistinguishable from linear, whereas those for neutron radiation are distinctly curvilinear, and can best be fitted to a power function with exponents of 0.6 to 0.75.

2) Fractionation of gamma radiation results in a longer mean survival time than is observed after the same total dose is given in a single exposure. Fractionation of neutron radiation, on the other hand, decreases the mean survival time relative to that observed at single doses. The difference is statistically significant at doses above 100 rads; as the total dose decreases, the dose-response curves for single and fractionated neutron exposures become essentially congruent.

3) The RBE for neutron radiation varies inversely with (approximately) the square root of the neutron dose over the ranges studied (5 to 240 rads, single exposures; 10 to 320 rads, 24 weekly fractions; 0.67 to 2.67 rads/weekly fraction, duration-of-life exposures).

Experiments are in progress (designated by JM number) that will extend and confirm the above mentioned findings, as follows:

1) JM-9 consists of single doses of 1, 2.5, 5, 10, 20, and 40 rads of neutron radiation and 22.5, 45, and 90 rads of gamma radiation. This experiment was designed to enable us to decide whether the neutron dose-response curve for life shortening becomes linear at some point below approximately 20 rads, or whether it continues to conform to the power function that is well fitted to the data from 5 to 240 rads. Of the 3950 mice committed to the experiment, only 20% have died; nevertheless, the data are developing in a manner consistent with our previous experience.

2) JM-10 involved the exposure of Peromyscus leucopus to 90, 143, 206, and 417 rads of gamma radiation, and 20, 40, 80, and 160 rads of neutron radiation in single doses, plus neutron exposures of 40 and 160 rads given in 24 weekly fractions. About one third of the animals are still alive. Provisional dose-response curves suggest that Peromyscus are more sensitive to neutrons than the $B6CF_1$ mice by a factor of about 2.5, based on days of life-span lost per rad, or a factor of 1.5, based on percent of life-span lost per rad. However, the radiation-specific excess mortality rates are approximately the same, and the two species show similar relationships between neutron RBE and neutron dose.

3) JM-12 studies the effects of neutrons given in 2, 4, or 6 fractions at weekly intervals, compared with the total dose (240 rads) given in a single exposure. Approximately two thirds of the mice are dead. The data confirm the augmentation phenomenon previously observed after a fractionation regime involving 24 or 60 weekly exposures.

4) JM-13 is a new experiment, initiated in February 1981, in which mice receive 60 once weekly exposures to neutrons (0.033 to 0.67 rads per fraction, total doses 2 to 40 rads) or gamma rays (1.67 to 10 rads per fraction, total doses 100 to 600 rads). Like the JM-9 series, this experiment should establish the shape of the dose-response curves at low dose rates and total doses. Both somatic and genetic end points are employed in this study; some results on genetic effects have already been obtained (see Section 6).

Meanwhile, we are continuing to analyze all data on specific causes of death as the results of histopathologic examination of the tissues of the decedents become available. The incomplete data suggest that the principal contribution to excess mortality after gamma irradiation comes from lymphoreticular

tumors; whereas after neutron radiation, lung tumors and tumors of other epithelial tissues are the principal contributors.

In conclusion, data developed in this program will allow the construction of dose-response curves for somatic effects (life shortening from all causes and several specific causes of death) in two rodent species, for single and fractionated exposures to both high-LET and low-LET radiation, down tc dose levels that are only an order of magnitude or so greater than the lifetime background exposure of control animals.

PUBLICATIONS, 1981 THROUGH APRIL 1982

Published

A Direct Data Entry System for Toxicologic Pathology
D. E. Doyle, L. S. Lombard, and T. E. Fritz
J. Toxicol. Pathol. 9, 22-29 (1981)

Life Shortening in Mice Exposed to Fission Neutrons and Gamma Rays.
I. Single and Short-Term Fractionated Exposures
J. F. Thomson, F. S. Williamson, D. Grahn, and E. J. Ainsworth
Radiat. Res. 86, 559-572 (1981)

Life Shortening in Mice Exposed to Fission Neutrons and Gamma Rays.
II. Duration-of-Life and Long-Term Fractionated Exposures
J. F. Thomson, F. S. Williamson, D. Grahn, and E. J. Ainsworth
Radiat. Res. 86, 573-579 (1981)

3. RADIATION TOXICITY IN DOGS

T. E. Fritz, L. S. Lombard, C. M. Poole, T. M. Seed, D. V. Tolle, J. M. Angerman, S. M. Cullen, D. Doyle, L. V. Kaspar, W. G. Keenan, A. R. Sallese, K. Jacaruso,* and L. Lanning+

The basic objective of this program is the generation of comprehensive data on the late effects of low doses of ionizing radiation in a large, relatively long-lived animal, the dog, that will be useful for extrapolation to man. The specific goals are: (1) to determine the influence of two major factors of exposure - the daily exposure rate and the accumulated total exposure; (2) to provide data for estimates of radiation-specific excess mortality in the dog to enable interspecies comparisons to existing rodent data and for development of a unifying concept of damage that has predictive value in man; and (3) to provide base-line data and specimens to characterize the pathogenesis and mechanisms of induction of leukemia, aplastic anemia, and other disease processes including late occurring soft tissue tumors.

These experiments utilize protracted whole-body ^{60}Co gamma irradiation given to young adult beagles 22 hours/day, 7 days/week. They characterize the responses at various exposure rates given either until death (0.4, 1.0, and 2.5 R/day) or until predetermined total exposures of irradiation are accumulated (600, 1400, 2000, and 4000 R). The latter are delivered at exposure rates of 5, 10, 17, and 35 R/day to compare with dogs previously irradiated until death at these exposure rates. The dogs are maintained for their entire life-span, and are monitored and evaluated at regular intervals by hematologic, clinical, and pathological examinations, and a complete necropsy is performed at death. All data are entered into a computerized data base.

Responses of young adult beagles to continuous exposure to ^{60}Co gamma rays. We have continued to monitor the dogs being irradiated continuously at 2.5, 1.0, and 0.4 R/day. As of May 1, 1982, these dogs had been irradiated for times varying from 1204 to 2264 days after their phasing into the experiment at 400 days of age.

Since last year, an additional 11 dogs have died for a total of 17 decedents, 16 experimental dogs and 1 control. Among the experimental groups, there have now been a total of 10 deaths in the 2.5 R/day group, 2 in the 1.0 R/day group, and 4 in the 0.4 R/day group. None of the deaths in the latter group are believed to be related to the irradiation (one case of encephalitis, two cases of a herniated vertebral disc, and one case of septicemia secondary to mastitis). The dead control dog died with signs of central nervous system disease related to cerebellar hypoplasia, a develop-

*Summer 1981 participant in the Undergraduate Honors Program, Rochester Institute of Technology.

+Participant in the 1981 Summer Biology Research Institute.

mental anomaly. Eight of the deaths in the 2.5 R/day group were related to the hematopoietic system; 6 were leukemias, 1 was a mast cell tumor, and 1 was an aplastic anemia. One of the remaining two dogs died of virus enteritis and the other of hemorrhagic myelitis.

Among the dogs still alive in all three exposure groups, damage to the hematopoietic system, manifested as depressed circulating blood cell levels, continues to be the only clinically detectable effect of the irradiation, except for testicular atrophy and aspermia.

As reported last year, there was early depression of thrombocyte and leukocyte values in all three exposure groups, but erythrocyte values remained stable for more than 1000 days, even at the highest exposure rate. There are exposure rate-dependent differences in the peripheral blood values for the three groups. Last year, we pointed out that these differences disappear for the three groups when the blood values are plotted against total accumulated exposure rather than against time of exposure. However, during the past year, the additional data obtained with continuing irradiation tend to show that values for both thrombocytes and leukocytes are stabilizing at differing exposure rate-dependent levels when the data are plotted against the greater accumulated total dose. At 600 R total exposure and greater, the leukocyte values for the 0.4 R/day group remain higher than those for the 1.0 and 2.5 R/day groups, and at 1200 R total exposure and greater, the leukocyte values for the 1.0 R/day group remain higher than those in the 2.5 R/day group.

Influence of dose rate and total dose on late effects of terminated exposures of young adult beagles to ^{60}Co gamma rays. The dogs given terminated exposures to predetermined total doses range between 2082 and 5298 days of age as of May 1, 1982. Although only 90 of 257 chronic survivors (35%) have died in these experimental groups, several interim observations and tentative conclusions can be made.

Myelogenous leukemia occurs at total exposures greater than 2000 R, and at exposure rates of 5, 10, and 17 R, but not at 35 R/day. These limits are obviously related to the facts that total doses above 1400 R given at 35 R/day are acutely lethal, and total exposures of over 2000 R are required to produce myelogenous leukemia in dogs irradiated continuously. Myelogenous leukemia for terminated as well as continuous irradiation occurs as the earliest occurring malignancy. The incidence of myelogenous leukemia in dogs given terminated exposures is less than that in dogs irradiated continuously, although irradiation continued beyond a certain time or total dose may not influence the incidence. This latter possibility is supported by the lack of leukemias in the longest term survivors at 10 R/day following the early wave of leukemias. The incidence of lymphocytic leukemia, in contrast to myelogenous leukemia, has not been affected by these terminated exposures, but their time of onset is significantly advanced. Similarly, most soft tissue tumors are occurring at significantly earlier times than in control decedents. In the case of mammary tumors, both the malignant and the benign forms are occurring at earlier times than their counterparts in the control group. The increased total number of malignancies over the control is attributable to four specific tumor types, myelogenous keukemia, nerve sheath and vascular sarcomas, and mammary tumors.

In summary, for the continuous exposures, the data to date support the hypothesis that the biological responses below 5 R/day are related to total dose rather than dose rate, as previously shown for life shortening in mice. For the terminated exposures, the limited data thus far available suggest that certain specific tumor types are occurring in numbers in excess of those observed in the control groups and at earlier times. Observation and monitoring of all dogs will continue, in order to provide data for further comparisons with the shorter lived laboratory species. These studies will help achieve our overall goal of assessing the hazards and understanding the mechanisms of radiation damage to man.

PUBLICATIONS, 1981 THROUGH APRIL 1982

Published

Viral Antibody Studies of Laboratory Dogs with Diarrheal Disease
L. N. Binn, R. H. Marchwicki, E. H. Eckermann, and T. E. Fritz
Am. J. Vet. Res. 42, 1665-1667 (1981)

A Direct Data Entry System for Toxicologic Pathology
D. E. Doyle, L. S. Lombard, and T. E. Fritz
J. Toxicol. Pathol. 9, 22-29 (1981)

Autoimmune Diseases of Canines
T. E. Fritz and N. R. Rose
The Canine as a Biomedical Research Model: Immunological, Hematological, and Oncological Aspects, M. Shifrine and F. D. Wilson, eds., 1980, Chap. 11, pp. 231-243

Radiosensitivity and Life Span of Dog Peripheral Blood Lymphocytes
A. Leonard, G. Decat, and T. E. Fritz
Mutat. Res. 92, 257-263 (1982)

In Press

Hematologic Responses of Beagles Exposed Continuously to Low Doses of ^{60}Co Gamma Irradiation
T. E. Fritz, D. V. Tolle, D. E. Doyle, T. M. Seed, and S. M. Cullen
Experimental Hematology Today/1982, S. J. Baum and G. D. Ledney, eds., Springer-Verlag, NY

Histologic Typing of Liver Tumors of the Rat
C. H. Keysser, L. S. Lombard, R. J. Montali, H. L. Stewart, and G. Williams
J. Natl. Cancer Inst.

Leukemia Induction in Beagles Exposed Continuously to ^{60}Co Gamma Irradiation: Hematopathology
D. V. Tolle, T. E. Fritz, T. M. Seed, S. M. Cullen, L. S. Lombard, and C. M. Poole
Experimental Hematology Today/1982, S. J. Baum and G. D. Ledney, eds., Springer-Verlag, NY

4. RADIATION-INDUCED HEMOPATHOLOGIES

T. M. Seed and L. V. Kaspar

Aplastic anemia and myelogenous leukemia occur in man as "spontaneous" diseases as well as following human exposure to radiation and chemical toxicants (e.g., benzene). This project uses the dog exposed to protracted gamma radiation to study the early changes in bone marrow structure and function involved in the prepatent stages of these two hemopathologies. Characterization of the early cellular events involved in the initial stages of aplastic anemia and leukemia is important in order to determine criteria for early diagnosis and prognosis and perhaps to develop preventive measures.

The objectives of the project are to (1) define the nature of pathological progression of both aplastic anemia and myeloid leukemia in beagles continuously exposed to low daily doses of gamma irradiation; and (2) elucidate cellular mechanisms of hematopoietic accommodation under chronic low dose irradiation, and the relationship between the accommodation and the induction and development of the major hemopathological end points.

The experimental approach is to assess serially the phase-related changes within the hematopoietic system (bone marrow) of beagles continuously exposed (22 hours/day) to low daily doses (10 R/day) of gamma radiation. Assessment employs both in vitro and in vivo assays aimed at compartmentalizing the granulopoietic system. The structure of biopsied marrow is evaluated by combination light and electron microscopy. Quantitative determination of absolute marrow cellularity is being performed by cell counts of total nucleated cells of marrow isolated from biopsied ribs. The major marrow compartments presently being examined include: (1) the granulocyte-monocyte "committed" stem cells (GM-CFUa), (2) granulocyte reserves, and (3) stromal elements.

Our earlier work demonstrated that hematopoietic recovery and accommodation of the gamma irradiated dogs occur following an initial period of hematopoietic depression. This recovery and accommodation are prerequisite to the onset of myeloid leukemia.

Four preclinical stages of radiation-induced myeloid leukemia have been identified and partially characterized: (I) the initial radiotoxic period; (II) partial recovery; (III) subnormal hematopoietic accommodation; and (IV) cytologically defined preleukemia.

The structural analysis of marrow during the hematopoietic recovery and accommodation phases (II and III) indicate a temporal, perhaps causal, relationship between the induced osteogenic-endosteal changes, stroma, and parenchymal restructuring on the one hand and the subsequent regenerative hematopoietic process on the other.

Characteristic changes in hematopoietic function during the late preclinical phases (late III and IV) include: (1) progressive, secondary increases in absolute marrow cellularity and in mobilizable granulocyte reserves that have a higher immature cell content; and (2) a marked decline in concentration of marrow GM-CFUa with a concomitant increase in aberrant progenitors having markedly reduced proliferative capacity.

The aberrant in vitro growth patterns of myeloid leukemia cells from 12 cases have been characterized. The most striking feature observed was a "lawn-type" growth pattern (an abnormally high number of single cells or small clusters of cells), which occurred in 10 of the 12 cases.

Based on experiments testing the radiation sensitivity of hematopoietic stem cells, derived at intervals from the marrow of the continuously irradiated dogs, a working hypothesis has been generated. The hypothesis attempts to relate changes in radiation sensitivities of hematopoietic progenitors under chronic gamma irradiation with predisposition to induced hemopathologies and to survival times. It is based on data that suggest an increased radioresistance of the granulopoietic progenitors, the suspected targets for the leukemogenic effects of whole body, low dose gamma irradiation.

The contributions of modified cell-cycle kinetics and repair functions in eliciting the change in radiosensitivity of the hematopoietic progenitors are presently under evaluation. Initial results of the analysis of cell cycle kinetics indicate that modification of the kinetics makes a minimal contribution to enhanced radioresistance.

In summary, preclinical phases of both radiation-induced aplastic anemia and myeloid leukemia have been partially characterized in terms of phase-specific changes in marrow architecture and for selected functional changes in various marrow cell compartments including the hematopoietic stem cells.

Prominent early cellular indicators of pathological predisposition to aplastic anemia, in contrast to the myeloproliferative disorders (e.g., myeloid leukemia), include: (1) the failure to initiate osteogenic-dependent repair processes involved in hematopoietic regeneration, and (2) a failure of the vital hematopoietic progenitors to develop increased radioresistance, which would lead to the onset of partial hematopoietic recovery (phase II of the sequence leading to leukemia).

PUBLICATIONS, 1981 THROUGH APRIL 1982

Published

Antiviral Properties of Polyinosinic Acids containing Thio and Methyl Substitutions
E. W. Chan, C. K. Lee, P. J. Dale, K. Nortridge, S. S. Hom, and T. M. Seed
J. Gen. Virol. 52, 291-299 (1981)

Cell Shape in the Alga Pediastrum (Hydrodictyaceae; Chlorophyta)
W. F. Millington, G. T. Chubb, and T. M. Seed
Protoplasma 105, 169-176 (1981)

In Press

Hematologic Responses of Beagles Exposed Continuously to Low Doses of ^{60}Co Gamma Irradiation
T. E. Fritz, D. V. Tolle, D. E. Doyle, T. M. Seed, and S. M. Cullen
Experimental Hematology Today/1982, S. J. Baum and G. D. Ledney, eds., Springer-Verlag, NY

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T. M. Seed, G. T. Chubb, and D. V. Tolle
Scanning Electron Microscopy/1981, O. Johari, ed., SEM, Inc., AMR O'Hare, IL

Early Effects of Castration and Replacement of Androgen on the Expression of Retrovirus-Like Particles in Rat Ventral Prostate Epithelial Cells
T. M. Seed, M. Rubenstein, and K. M. Anderson
Proc. Soc. Exp. Biol. Med.

Leukemia Induction in Beagles Exposed Continuously to ^{60}Co Gamma Irradiation: Hematopathology
D. V. Tolle, T. E. Fritz, T. M. Seed, S. M. Cullen, L. S. Lombard, and C. M. Poole
Experimental Hematology Today/1982, S. J. Baum and G. D. Ledney, eds., Springer-Verlag, NY

5. RADIATION BIOLOGY OF CULTURED MAMMALIAN CELLS

A. Han, M. M. Elkind,* E. Ben-Hur,+ F. Buonaguro, C. K. Hill, H. Utsumi,α F. Suzuki, E. M. Buess, J. L. Dainko, P. J. Dale, and C. M. Liu

The primary objective of this project is the elucidation of the mechanisms of radiation-induced changes in mammalian cells that lead to cell lethality, mutation, and neoplastic transformation. The influence of repair processes on these end points is of particular interest. Radiations used include different qualities of ionizing radiations and nonionizing radiations of different wavelengths.

The principal techniques that are used include clonal growth of single cells to measure cell lethality or induction of mutants, labeling of macromolecules with radioisotope-labeled precursors, chemical identification of protein and nucleic acids components, and measurement of damage to DNA using sedimentation and elution techniques. In studies of neoplastic transformation in vitro, tests for altered properties are made by measuring the ability of cells to grow in semisolid agar, production of plasminogen activator at higher levels, and tumorigenicity of transformed cells after their inoculation into suitable hosts.

Neutron radiobiology of mutation and neoplastic transformation. To increase our understanding of neoplastic transformation and the dependence of radiation quality and dose rate on the process, experiments were carried out with C3H10T1/2 cells using reduced dose rates of JANUS fission neutrons. This beam is quite similar to the principal radiation of concern in the nuclear power industry. Thus far, the results are limited to a comparison between neutron irradiation delivered at high dose rates of 10 to 38 rads/minute, and low dose rates of 0.43 and 0.086 rad/minute. The data show that, over the range of low total doses from 10 to 100 rads, the frequencies of neoplastic transformation at a low dose rate are significantly higher than those after the same exposure delivered at a high dose rate. However, in the higher dose range (above 150 rads), incidences of transformation are about the same regardless of the rate of neutron delivery.

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Induction of mutants resistant to 6-thioguanine by JANUS neutrons and low-LET radiations (X-rays and ^{60}Co gamma rays) was measured using V79 Chinese hamster cells. The induction curves for low-LET radiations appear linear, and can be fitted with one line for both X- and gamma-rays. For JANUS neutrons, the initial region of the induction curve is linear, but then the curve tends to level off at doses greater than 150 rads. These preliminary data suggest a constant RBE of about 9 for mutation induction by fission spectrum neutrons at the hypoxanthine-guanine phosphoribosyltransferase locus.

^{60}Co gamma-ray dose protraction and dose fractionation: The effect on neoplastic transformation. Exposure of C3H10T1/2 cells to ^{60}Co gamma rays at the low dose rate of 0.1 rad/minute results in significant reduction in the incidence of neoplastic transformation compared with delivery of the same total doses at a high dose rate (100 rads/minute). At both rates of delivery, the initial part of the induction curve is linear, and at 0.1 rad/minute the slope is about half as steep as it is at 100 rads/minute. Thus, the results suggest that cells can repair the subtransformation damage in the linear, "single-hit" portion of the curve.

Further studies relative to the repair of subtransformation damage show that irradiation of exponentially growing cells to total doses of 25 to 300 rads of ^{60}Co gamma rays, in five equal daily fractions, results in a significant reduction in transformation frequencies compared with single doses at 50 rads/minute. Further reduction is indicated when the interfraction interval was increased from 24 to 48 hours.

Interaction of radiations of different qualities: Mutation and neoplastic transformation. A conditioning dose of UVB light (290-245 nm), given immediately before exposure to UVC light (254 nm) results in a significant inhibition of induction of Chinese hamster V79 cells resistant to 2 mM ouabain by the UVC light. The inhibition is lost, however, if the cells are incubated for 12 hours at 37°C between irradiations. Inhibition is also observed when cells are preexposed to a dose of UVB filtered with polystyrene (300-345 nm) which, in itself, has no effect on cell killing. Conditioning exposures of UVB light, unfiltered or filtered, have no effect on the induction of 6-thioguanine-resistant mutants by UVC light. These observations imply that the long wavelengths inhibit an error-prone repair mechanism that might be related to the induction of point mutations in mammalian cells.

Synergistic interaction between UVC light and X-rays in induction of neoplastic transformation occurs when 10T1/2 cells are exposed to a small dose of UVC light prior to X-irradiation. However, the effect is significantly smaller if UVC irradiation is delivered after X-rays. Although the interaction is synergistic in induction of neoplastic transformation, it is additive for cell lethality, thus suggesting that the mechanisms of damage expression for these two end points might be different.

Repair of single-hit lethal damage. Chinese hamster V79 cells in late S-phase were exposed to low doses of JANUS fission spectrum neutrons, and the influence of postirradiation treatment with anisotonic buffer, medium containing 90% D_2O, or medium with 2 mM caffeine on survival was examined.

Neutrons rather than X-rays were used because the initial slope of the neutron survival curve is appreciably steeper. The data indicate that, for each treatment, the initial slope is increased in repair-competent cells. The inference follows that "single-hit" lethal damage after high-LET irradiation is ordinarily repairable in repair-competent cells.

In conclusion, the foregoing studies with mammalian cells, with both ionizing and nonionizing radiations, indicate the important role of repair processes in expression of radiation-induced damage. The studies are being extended to examination of molecular mechanisms, and DNA damage and repair in particular. Finally, our finding that the incidence of transformation is increased at low dose rates of neutrons, compared to acute exposure, implies that the risk of cancer induction due to work related exposures in the nuclear power industry may be greater than expected.

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6. MAMMALIAN GENETICS

D. Grahn, M. L. Garriott, B. H. Farrington, C. H. Lee, J. J. Russell, and D. J. Crowe*

The objectives of this program are threefold: (1) To assess genetic hazards from testicular burdens of ^{239}Pu and determine retention and microdistribution of this transuranic element in the testis. (2) To compare effects of ^{239}Pu with single, weekly, and continuous ^{60}Co gamma irradiation and single and weekly fission neutron irradiation to develop a basis for estimating relative biological effectiveness (RBE) for genetic end points. (3) To develop detailed dose-response data for genetic end points of concern at low doses of neutrons and gamma rays.

Comparatively short-term genetic end points are used, namely: (1) the dominant lethal mutation rate in both spermatogonial and postspermatogonial cell stages, (2) the frequency of abnormal sperm head morphology measured at various times after irradiation, (3) the frequency of reciprocal chromosome translocations induced in spermatogonia and measured at first meiotic metaphase, and (4) the frequency of chromatin fragments (micronuclei) in the polychromatophylic erythrocyte in the bone marrow. Male hybrid $B6CF_1$ mice, 120 days old, are used for all studies. Measures of the retention, microdistribution, and microdosimetry of plutonium in the testes are obtained by conjoint histological and autoradiographic procedures after intravenous injection of monomeric plutonium citrate. Other males are exposed to external fission neutrons from the JANUS reactor and to ^{60}Co gamma radiation. Breeding tests are performed with the irradiated males at selected periods to obtain data on postmeiotic, meiotic, and premeiotic cell stages in male gametogenesis.

Single neutron doses of 1, 2.5, 5, 10, 20, and 40 rads have been compared with the effects of 22.5, 45, and 145 rads of ^{60}Co gamma rays, using the same genetic end points employed in the plutonium studies. A weekly, long-term exposure series has also been performed for additional neutron and gamma ray comparisons at low weekly increments of 0.67, 1.67, and 2.67 neutron rads vs. 6.95, 17.4, and 32.0 gamma rads. Males were periodically screened for accumulation of dominant lethal mutations and for the frequency of chromosome aberrations.

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Genetic effects of gonadal burdens of plutonum. The measurement of translocation frequencies induced over a 60-week period by weekly neutron and gamma ray exposures compared with continuous alpha particle irradiation confirms previous observations that the response to ^{239}Pu alpha particles is nonlinear (concave downward) and falls below the neutron-induced response. The nonlinear response probably results from the heterogeneous deposition of the alpha emitter which permits some spermatogenic clones to remain undamaged and to progressively become the major source of differentiating germ cells.

A study has been initiated to estimate ^{239}Pu alpha particle dose to the different germinal elements in the C3H X 101 F_1 hybrid male used by the Oak Ridge National Laboratory. The study is part of a continuing collaboration with the Mammalian Genetics Program which is performing a specific genetic locus mutation rate assay to estimate the rate of induction of dominant mutations causing abnormal skeletal development.

Genetic effects of the external radiations. The rate of induction of translocations by gamma rays is significantly reduced by fractionation, reduced weekly dose level, and reduced dose rate, as noted by others. Weekly fractions of neutrons are more mutagenic than a single exposure at total doses above 20 rads, and the response is linear. In contrast, the response to single neutron exposures is markedly nonlinear. There is no variation in level of response attributable to variation in the size of the weekly neutron dose over a 20-fold range from 0.67 to 13.33 rads/week.

In the extremely low single dose studies, there is a continuous, essentially linear response for the induction of dominant lethal mutations in postspermatogonial stages down to the lowest doses. There is a slight but not significant response in excess of predicted levels, which is not greater than a factor of two above expectation. The RBE at 1 to 5 rads of neutrons is no greater than 10. For translocations, there is also the hint of response in excess of expectation for neutron irradiation, and the RBE at low single doses appears to be between 5 and 10. In comparison with low intensity continuous gamma irradiation, the RBE is no less than 35.

Weekly gamma and neutron irradiations induce dominant lethal mutations at a predictable rate in general conformance with expectation; however, there is a 1-1/2 to 2-fold excess of lethal mutations induced in the postmeiotic germ cells exposed to weekly neutron exposures.

A major long-term study initiated in February 1981 concerns both somatic and genetic effects of low once weekly exposures to fission neutrons or ^{60}Co gamma rays. The somatic effects are being studied in Experiment JM-13 under Neutron and Gamma-Ray Toxicity Studies (see Section 2). Sixty weekly doses are being delivered to both sexes of the B6CF_1 hybrid mouse at six neutron dose rates ranging from 0.033 to 0.67 rads/week and five gamma ray levels between 1.67 and 10 rads/week. Samples are being taken during the 60-week exposure period to measure both transient and cumulative genetic injury after 10, 25, 40, and 60 exposures. Genetic end points include the frequencies of abnormal sperm morphology, reciprocal chromosome translocations, and micronuclei. Dominant lethal mutation rates are being estimated in the intervals of 11 to 22 exposures and 41 to 52 exposures. Results to date generally confirm the occurrence of genetic damage at levels consistent with expectations derived from prior studies at higher levels of weekly exposure.

In summary, genetic investigations with plutonium are essentially complete. The unpredictable and nonlinear incidences of chromosome translocations observed as a function of alpha particle dose have been confirmed. The radiobiological basis of the nonlinear response is concluded to be due to the intrinsic heterogeneity of ^{239}Pu deposition in the gonad, in combination with the random positioning of stem cell clones in the tubules.

Weekly exposures to low level neutron or gamma radiations, for periods up to 45-50 weeks, induce dominant lethal mutations at a rate that generally conforms to a simple model based on differences in the sensitivities of the different germ cell stages to mutation induction. There is a consistent excess over expectation in the frequency of dominant lethal mutations induced by neutrons as compared to gamma rays; which is demonstrated to have been induced in the postmeiotic and meiotic stages. Translocations, which are induced in the stem cells, occur as a linearly increasing function of accumulating dose of either neutrons or gamma rays and do not show the deviations from linearity noted above for dominant lethal mutations.

CARCINOGENESIS

7. MECHANISMS OF HEPATOCARCINOGENESIS

C. Peraino, J. E. Morris,* W. E. Boernke,+ V. A. Ludeman,
A. M. Prapuolenis, and E. F. Staffeldt

This project is directed toward the analysis of mechanisms of tumor initiation and promotion, using rat liver as the model system, and the development of the liver system as a method for the rapid screening of a broad range of environmental pollutants and other chemicals for tumor initiating or promoting activity. Part of our investigation of tumorigenesis mechanisms involves the study of the control of gene expression in liver, using ornithine aminotransferase (OAT) and serine dehydratase (SDH) adaptation as the experimental model. Studies of the self-associative properties of the OAT molecule are designed to provide insight into the relationship between these properties and the regulatory function of this mitochondrial enzyme in the intact organism.

In the tumorigenesis studies, newborn rats are injected with tumor initiators and fed tumor promoters beginning at the time of weaning; at intervals thereafter the livers are examined for the appearance of altered hepatocyte foci and tumors. Serial frozen liver sections are assayed histochemically for the appearance of foci containing one of seven alterations. By superimposing the images of the successive sections it is possible to determine the numbers and sites of foci containing various combinations of histochemical markers with the aid of an image digitizer and computer.

In the OAT and SDH regulation studies, rats exposed to adaptive stimuli (hormonal treatments, changes in feeding and lighting schedules) are injected with ^{3}H-leucine prior to sacrifice. The ratio of antibody-precipitable radioactivity to total acid insoluble radioactivity is used as a measure of the relative rate of synthesis for each enzyme. Self-associative properties of OAT are examined using enzyme preparations purified to crystallinity by a procedure devised in this laboratory.

With our new tumorigenesis treatment protocol, histochemically altered foci appear within one week after weaning in rats given diethylnitrosamine (DEN) at birth and weaned onto a diet containing promoter; tumors appear within eight weeks. Administration of benzo(a)pyrene (BAP) at birth followed by promoter at weaning produces foci within 3 weeks after weaning and tumors within 16 weeks; for both carcinogens females appear to be substantially more responsive than males.

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+Visiting Scientist, Nebraska Wesleyan University.

7. MECHANISMS OF HEPATOCARCINOGENESIS

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The relative frequencies of the various types of histochemically altered foci produced in rats receiving DEN or BAP are similar, although the rate of appearance and overall incidences of foci and tumors is considerably lower in the BAP-treated rats compared with those given DEN. These findings suggest that the two carcinogens, though differing widely in chemical properties, pathways of metabolism, and hepatocarcinogenic potencies produce the same spectrum of changes in the preneoplastic liver foci.

In our prior report, we indicated that the circadian cycling of OAT and SDH synthesis rates were 12 hours out of phase in rats maintained under constant light and conditioned to eat their entire daily ration in a single 2-hour interval. Our current results indicate that whereas the SDH cycling is highly consistent, the phasing of the OAT cycling in the same animals shows considerable drift (sometimes in phase and sometimes out of phase with the SDH cycle). The apparent absence of a consistent relationship in the phasing of the OAT and SDH synthesis cycles suggests that the mechanisms regulating the circadian cycling of OAT and SDH synthesis are completely independent.

Our sedimentation equilibrium studies showed that OAT aggregates in a two-stage process as its concentration increases. The first stage involves the association of enzymatically active monomers into trimers, with association of the trimers into higher order aggregates occurring in the second stage. Kinetic analyses of purified enzyme showed that aggregation results in increased apparent Michaelis constants (K_m's). Increased K_m values were also obtained for OAT sequestered in mitochondria from rats fed a high-protein diet to increase mitochondrial OAT levels. The higher K_m values suggest that the elevation of OAT *in vivo* is accompanied by aggregation of the enzyme within the mitochondrion.

In conclusion, the sensitivity, rapidity, and simplicity of our tumorigenesis protocol suggest that it will significantly enhance the utility of liver tumorigenesis as an *in vivo* bioassay for detecting environmental carcinogenesis and will also facilitate the analysis of the sequential changes occurring in hepatocytes during the onset of neoplasia. The major implication of our histochemical findings is that the spectrum of foci that we have observed represents a generic preneoplastic response that is independent of the nature of the carcinogen administered. Consequently, the thorough investigation of the relationship among the various types of foci should provide a detailed picture of stages of hepatic neoplasia from the earliest detectable manifestation to the final appearance of tumors.

Our data on OAT self association suggest that the aggregation of OAT *in vivo* has physiological significance in that this process reduces ornithine degradation, thereby sparing it for the maintenance of urea cycle activity. Therefore, the reversible concentration-dependent polymerization of OAT in effect serves as a damping mechanism for preventing excessive and rapid fluctuations in the ornithine pool that might be produced by changes in OAT levels.

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8. MUTAGENESIS AND CELL DIFFERENTIATION IN CHEMICAL CARCINOGENESIS

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I. Simon, M. F. Callaham, C. B. Henning, and B. A. Sedita

Our studies are directed toward the development of short-term assays for the identification of chemicals that can act as tumor initiators and/or promoters, as well as toward the understanding of the processes that bring about initiation and promotion. To avoid the complexity in studying chemical carcinogenesis in whole animals, we employ human and rodent cell culture systems and use alterations in cell differentiation and mutagenesis processes as end points.

Mutagenesis studies employ our previously developed cell-mediated assay in which suitable target cell types that cannot metabolize many chemicals are cocultivated with intact normal cells that can metabolically activate chemical carcinogens.

Using the cell-mediated assay, we established a quantitative relationship, for more than 30 nitrosamines, between mutagenic activity and carcinogenic effectiveness. In this case, mutation of Chinese hamster V79 cells to ouabain and 6-thioguanine resistance was measured when the cells were cocultivated with primary cultures of Fisher rat hepatocytes, capable of activating nitrosamines. This study demonstrates that the use of intact hepatocytes to activate nitrosamines, coupled with appropriate mammalian target cells to indicate genetic damage, is a useful system both for detecting and establishing the mutagenicity of a series of nitrosamines in a manner corresponding to the carcinogenic potency of the same chemicals in vivo. In a fibroblast-mediated assay we have previously established a similar correlation between the mutagenicity and the carcinogenicity of polycyclic hydrocarbons. We plan to extend these studies to investigate species and organ-specific differences in chemical carcinogenesis by using the hepatocytes or epithelial cells from colon and lung from different species as both activating and target cells.

To increase the spectrum of markers available for use in cell-mediated assays, we developed in V79 cells a procedure for a one-step selection of cell variants resistant to the cytotoxic effect of mycophenolic acid, an inhibitor of inosine monophosphate (IMP) dehydrogenase (IMP:NAD^+ oxidoreductase, EC 1.2.1.14) an enzyme that is usually elevated in malignant cells. The

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frequency of these variants was increased in a dose-dependent manner after treatment with the mutagen/carcinogen N-methyl-N'-nitro-N-nitrosoguanidine and after an expression time of 8 days. The enzyme activity from both the variants and the parent cells had a similar affinity for the substrate IMP and a similar response to mycophenolic acid. However, the degree of resistance in the isolated cell variants was associated with a comparable increase in the specific activity of IMP dehydrogenase. We therefore suggest that such cell variants, which have an altered regulation of IMP dehydrogenase activity, may be a helpful tool in studying the control of cell growth and carcinogenesis, and may be useful as markers in short-term assays for the identification of potential chemical carcinogens.

The effect of tumor promoters was analyzed in a number of cultured human melanoma and leukemia cell lines, including HL-60 promyelocytic leukemia cells. These cells were chosen because they display useful markers of cell differentiation and enable the study of the mode of action of chemicals that can alter cell differentiation processes. Treatment of the HL-60 leukemia cells with the tumor-promoting phorbol diesters, including phorbol-12-myristate-13-acetate (PMA), caused terminal differentiation in these cells. Teleocidin, a nonphorbol diester tumor promoter similarly affects the HL-60 cells. Both the phorbol diesters and teleocidin cause changes in cell morphology, as well as increases in lysozyme and nonspecific esterase activity - characteristics associated with differentiation to macrophages. In addition, both compounds cause HL-60 cells to accumulate in G_1-phase at the expense of S-phase cells. We have been able to select an HL-60 cell variant (R-60) that is resistant to PMA-induced differentiation. None of the above described effects was observed in this resistant variant. These PMA-resistant cells are, however, as susceptible as the parent cells to induction of granulocyte-like cell differentiation by agents such as dimethyl sulfoxide and retinoic acid.

In other studies, we have shown that HL-60 cells can specifically bind phorbol diesters and that the PMA-resistant cells are defective in the "down regulation" of such a specific binding (i.e., a loss of cellularly bound phorbol dibutyrate following its maximal specific binding). This suggests that down regulation of specific receptors common to both the phorbol diesters and teleocidin may perhaps be an important factor in the induction of cell differentiation by phorbol diesters and related chemicals in HL-60 cells and perhaps other cell types.

Based on these studies, we suggest that certain chemicals that will produce effects similar to those of PMA and teleocidin in the susceptible and resistant HL-60 cells may be potential tumor promoters. Thus, this cell system and the cell-mediated mutagenesis assay constitute a valuable approach and tool for the detection and study of potentially carcinogenic chemicals in the environment.

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Proc. Natl. Acad. Sci. 78, 1062-1066 (1981)

*These publications are included here as they are directly relevant to this research program. They grew out of work performed at Oak Ridge National Laboratory under the direction of Dr. E. Huberman, prior to his transfer to Argonne National Laboratory as Director of the Division of Biological and Medical Research.

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Cancer Res.

9. MOLECULAR AND GENETIC MECHANISMS OF MUTAGENESIS

H. E. Kubitschek, P. R. Reynolds, and D. Williams-Hill

This program is primarily concerned with elucidation of the nature of the DNA lesions produced by environmental and energy related mutagens as well as their mechanisms of action, and their repair. Our main focus is on actions of chemical mutagens and electromagnetic radiations. Individual chemical mutagens, carcinogens, or agents producing lethal mutations are chosen for study on the basis that they are an important environmental hazard, which may prove to be a serious population risk, or that they represent a class of agents, examination of which is expected to expand our general knowledge of processes of mutation and repair. Cell growth during the cell cycle is also studied because little is known of the growth processes or their control, and because growth also affects the production and expression of mutagenic DNA lesions.

The conventional tools of microbial and molecular genetics are used in bacterial studies, along with intercomparison of genetically related strains. Advantage is taken of techniques for studying lesions such as pyrimidine dimers and DNA strand breaks in repair-competent and repair-deficient strains. The use of continuous culture techniques often provides special advantages. Mammalian cell cultures are also used in some collaborative studies.

Because near-UV is known to affect cell membranes and to produce single strand breaks (SSB's) in DNA, we have recently proposed that the near-UV target may be a DNA-membrane complex. Our preliminary results support this hypothesis. Cell survival and induction of SSB's by near-UV were compared in a fatty-acid requiring strain of E. coli K1060 after growth in the presence of the membrane-precursor fatty acids eicosenoic (one C:C double bond) or arachidonic acid (four C:C double bonds). Both killing and the number of unrepaired SSB's were enhanced with arachidonic acid, indicating that both the cell membrane and its DNA affected survival. (In collaboration with M. J. Peak and R. W. Tuveson.)

The chemostat B_{S-1} assay was examined for its sensitivity to potassium dichromate, as an indication of the ability of this testing system to detect metal mutagens. However, mutation rates in the presence of potassium dichromate were not significantly greater than in its absence.

During the year, a breakthrough was made on bacterial cell growth that may lead to new understanding of cell growth and its controls. In collaboration with C. L. Woldringh (University of Amsterdam), using data from electron micrography measurements of thousands of growing cells of E. coli, it was found that both cell volume and surface area increase bilinearly during the cell cycle, i.e., rates of increase are initially constant and double at some point durng the cycle. Because bacterial cell density is essentially constant during the cell cycle, bilinear volume increase requires a corresponding bilinear increase in cell mass. These results also agree with our earlier observations on cell mass and volume increase in bacteria and eucaryotic cells. The fact that very different kinds of cells have similar patterns suggests that very similar mechanisms exist for controlling cell growth. In addition, our analysis of bacterial growth provides the first evidence for closely linked biochemical pathways controlling increase in cell mass and surface enlargement. This is an unexplored area that may contain a key to our understanding of cell growth processes.

Preliminary evidence has been obtained that cell density is extremely constant during the cell cycle in suspension cultures of each of three different mouse cell strains (myeloma S107, and lymphomas 70 Z/3 and Abe8). Mean cell densities appear to be independent of cell age, with a standard deviation of about 0.3%. Such extreme constancy of these mammalian cell systems will allow the determination of cell mass from measurements of cell volume. (In collaboration with M. R. Loken, University of Chicago.)

In conclusion, our observations on cell growth may lead to a new understanding of cellular growth processes. Bilinear cell growth during the cell cycle and coordinate biochemical control of increase in cell wall and mass would require the operation of very specific control mechanisms, heretofore unexamined. Validation of coordinate, bilinear growth in eucaryotes would provide a major advance and open new directions in the study of cell growth and its controls.

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10. MOLECULAR AND CELLULAR EFFECTS OF SOLAR ULTRAVIOLET LIGHT

M. J. Peak, J. G. Peak, R. W. Tuveson,* M. A. Turner,+ and L. A. Nerad[α]

This program addresses the basic nature and mechanism of lethality, mutagenesis, and transformation caused by environmentally important nonionizing solar radiations (> 290 nm) present in our ecosystems. This research is currently of particular significance because of the possibility of enhanced fluxes of UVB (290-330 nm) from attenuation of stratospheric ozone due to release of halocarbons by man. The program addresses DNA damages leading to lethal and mutagenic effects, and the roles played by naturally occurring and experimentally added molecular probes in the mechanisms of these events.

Biological procedures used are largely bacterial genetic systems for biological end points, including isolated, genetically active transforming DNA and strains of bacteria differing in repair capability. Recently, the program was extended to include eucaryotic cell systems, mammalian cells in culture, and whole animal studies. Physicochemical end points include analysis of breakage of DNA *in vitro* and *in vivo* (alkaline sucrose gradient centrifugation), DNA-protein cross-linking (alkaline elution), and UV-induced pyrimidine dimers (endonuclease-sensitive sites). Our recent results include the following items.

The spectral analysis for the *in vivo* induction of DNA breaks in *Bacillus subtilis* (254, 290, 313, 334, 365, 405, and 434 nm) elucidated some properties of these breaks. For instance, about 80% of the breaks are true backbone scissions, the remaining 20% are alkali-labile lesions; oxygen is required for more than 90% of the breaks, and diazobicyclo(2.2.2.)octane and glycerol protect against the breaks. A completed action spectrum for breakage of DNA *in vitro* showed a greater susceptibility for breaks compared with DNA *in vivo*, possibly because of cellular repair processes in the latter.

Analysis of DNA breaks caused by 365-nm UV in two strains of *Escherichia coli* with specific sensitivity to near-UV killing (*nur*) showed that both the *nur* strains were more sensitive to DNA breakage induced by 365-nm UV than was the wild-type *nur*$^+$ parent. These experiments indicate that DNA breakage may be important for lethality caused by 365-nm UV. Several new *E. coli* mutants specifically sensitive to near-UV radiation were isolated,

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using a defective bacteriophage Mu(Amp lac) as the mutagen. These mutants will be valuable for identifying genes controlling cellular responses to near-UV and in future studies on their regulation.

Using strains of E. coli lacking 4-thiouridine, this molecule was shown to be an important chromophore in near-UV lethality. These strains are also refractory to near-UV induced DNA breakage, reinforcing our recent understanding of the potentially important role of DNA breaks in lethal events caused by near-UV. The possibility that 4-thiouridine may be a naturally occurring photosensitizer, acting in the form of the free nucleotide, is supported by the observation that isolated DNA is broken by 334 nm in the presence of 4-thiouridine added in vitro. Similar experiments using bacterial mutants indicated that menaquinone is not an important chromophore.

A spectrum for the protection by glycerol of transforming DNA against breakage caused by near-UV was completed, and matches closely the spectrum for protection of DNA by glycerol against inactivation of genetic activity by near-UV. This is evidence that inactivation of genetic activity by near-UV may be due to DNA breakage.

A detailed action spectrum for UVB lethality and mutagenesis in E. coli was completed, using 13 wavelengths, 8 of them in the UVB region. This spectrum enables quantitative predictions of the impact of attenuated stratospheric ozone upon mutational burden caused by increased fluxes of UVB. (In collaboration with R. B. Webb.)

Other collaborative studies have been particularly productive this year. The results of some of these are summarized below.

1) Induction of the synthesis of new proteins in E. coli by UV light was studied by slab-gel electrophoresis after irradiation at 254, 290, 313, and 365 nm UV. In rec^+ strains, all these wavelengths apparently induced a new protein which migrated to a position similar to that of the rec protein. (In collaboration with A. Eisenstark, University of Missouri, Columbia, and M. A. Turner.)

2) We have demonstrated, in the biological dose range, dose-dependent induction by monochromatic 365-nm UV of DNA-protein cross-links in Chinese hamster cells in culture. (In collaboration with A. Han.)

3) Mutagenesis by monochromatic 365-nm UV in human cells in culture at doses well within the biological range was demonstrated for the first time. (In collaboration with C. A. Jones.)

4) An action spectrum for the induction of pyrimidine dimers in the skin of hairless mice by UVB showed that maximum induction was by 293-nm UV, enabling the relative spectral transmittance of mouse skin to UVB to be derived. (In collaboration with R. D. Ley, Oak Ridge National Laboratory.)

5) Administration of arsenic in the drinking water modifies the induction of skin tumors by solar UV in hairless mice. Low levels of arsenic augment UV carcinogenesis and toxic levels inhibit it. (In collaboration with Y. E. Rahman; see Section 13.)

In conclusion, advances have been made that increase our understanding of molecular events caused by solar-UV. Especially significant is the implication of DNA breaks in lethal events caused by these radiations, and the identification of the role played by 4-thiouridine in these breakages. At the same time, careful spectral analyses are proving of value in prediction of increased hazards on biological systems in the event of increased fluxes of UVB. Inclusion of eucaryote cells in culture and whole animals has increased the relevance of this program.

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Photochem. Photobiol.

11. MECHANISMS OF DAMAGE AND REPAIR BY UVA, UVB, AND UVC

R. B. Webb

Recent work has been directed toward elucidating the molecular mechanisms of bacterial cell killing and mutagenesis by radiation in the UVB (290-320 nm), UVA (320-400 nm), and visible light (400-740 nm) ranges, in the absence of added photosensitizers. DNA breaks, inhibition of repair, and synergisms and antagonisms between radiation of different wavelengths are being studied for both cell killing and mutagenesis.

Approaches include the use of a large 2.5-kW monochromator of special design along with a variety of monochromatic and spectrally defined broad-spectrum sources, including a solar simulator. Calibrated radiometers, thermopiles, and a calibrated spectroradiometer are essential to these studies. Bacterial strains with different defined repair characteristics are used as they are appropriate to the specific investigation. Current techniques in photobiology, genetics, molecular biology, organic chemistry, and microbial culture are used as required.

Action spectra for *nur* and *nur*$^+$ strains of *E. coli* have been obtained. The *nur* gene produces a sensitizing effect at wavelengths from below 313 nm to 405 nm (the longest wavelength tested). The maximum effect was obtained at approximately 340 nm. Little or no effect was obtained below 290 nm. In addition, the *nur* trait sensitizes equally against monochromatic and broad-spectrum radiation in the 300-405 nm wavelength range. We propose that the *nur* trait affects a component of DNA repair that is specific for damage produced by UVA and UVB radiation. (In collaboration with R. W. Tuveson.)

Recent data clearly establish that the superficial similarity of lethal and mutagenic effects produced by UVC (200-290 nm) and UVB mask basic differences in the mechanism of action of these two wavelength ranges. Use of the highly sensitive *uvrA recA* strain of *E. coli* K12 AB2480 revealed significant differences in the kinetics of lethality and the lethal component subject to enzymatic photoreactivation. These and other data implicate different nondimer DNA lesions for cell killing in the UVC and UVB ranges. It is suggested that lethality in the UVB range involves components of both UVC and UVA effects. However, the presence of unique UVB mechanisms for lethality has not been ruled out.

It has been demonstrated that DNA lesions produced by an oxygen-dependent mechanism account for the major part of the cell lethality in bacteria by UVA in wild-type, uvrA, and recA strains. True single-strand breaks fulfill these requirements, and are produced in biologically significant frequencies. However, knowledge of the full range of oxygen-dependent and oxygen-independent DNA lesions produced by UVA and UVB remains seriously limited. Furthermore, the complex nature of oxygen dependence of UVA mutagenesis militates against a major role of single-strand breaks in the induction of mutations by UVA.

It has been shown previously that pyrimidine dimers are produced in the UVA range (up to 380 nm) at biologically important frequencies. These dimers clearly are a major factor in UVA mutagenesis and in cell killing in radiation-sensitive uvrA recA strains. However, dimers are not a significant factor in cell killing by UVA in wild-type and uvrA bacterial strains. Various kinds of evidence indicate that cyclobutyl pyrimidine dimers produced at 365 nm are repaired with much greater efficiency than dimers produced at 254 nm in wild-type and uvrA strains. This observation of the effective dark repair of dimers produced by 365 nm is consistent with the absence of detectable enzymatic photoreactivation in uvrA and wild-type strains by UVA. It is proposed that a nondimer DNA lesion produced by UVC may interfere with the maximum repair of dimers.

The work presented above, together with collaborative research not presented, can be summarized as follows:

The nur trait uniquely sensitizes E. coli cells to wavelengths in the 300-405 nm range; the maximum effect occurs at 340 nm.

Lethal effects by UVB (290-320 nm) and UVC (200-290 nm) are produced by different mechanisms. These differences are largely masked in repair-proficient strains.

DNA lesions produced by oxygen-dependent processes clearly account for 70 to 95% of UVA lethality in all bacteria tested that are capable of DNA repair and in mouse myeloma cells. True single-strand breaks represent such oxygen-dependent lesions, but other DNA lesions have not been ruled out. Cyclobutyl pyrimidine dimers produced by UVA through a sensitization process (not enhanced by oxygen) are repaired with such great efficiency that they make no detectable contribution to cell lethality under aerobic conditions. However, dimers are implicated in mutagenesis resulting from high fluence rate UVA. Furthermore, membrane damage is largely reversible, and thus contributes little to UVA and UVB lethality.

Strong nonreciprocal interactions between UVA and UVC of both synergistic and antagonistic types have been demonstrated for both mutagenesis and lethality.

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T O X I C O L O G Y

12. METAL METABOLISM AND TOXICITY

M. H. Bhattacharyya, R. P. Larsen,* P. A. Benioff,+ H. C. Furr,α
E. S. Moretti, R. D. Oldham,* D. P. Peterson, M. I. Spaletto,* and
M. E. Shackelford**

The objective of this program is to gain new insights into the metabolism and toxicity of metal compounds in experimental animals and man. Chosen for study are metal compounds related to specific energy technologies, and exposure conditions relevant to human exposure. Three areas of study are identified: (1) metabolism and toxicity of nonnuclear toxic metals, (2) gastrointestinal absorption of actinide elements, and (3) assessment of health and environmental effects of battery energy storage technologies. The latter study, covered in earlier Annual Reports, is not updated here.

Methods used include radioactive tracer techniques to determine pathways for uptake and tissue distribution of cadmium (^{109}Cd) and lead (^{210}Pb) during pregnancy and lactation in mice. Solutions are administered either continuously via drinking water or by gavage; tissue samples are analyzed by gamma spectrophometry.

In toxicity studies utilizing compounds of stable elements, metal concentrations are determined by flame or flameless atomic absorption spectrophotometry (lead, cadmium, arsenic) or by UV-visible spectrophotometry (antimony). Toxic responses under study include alterations in kidney and skeleton for cadmium; in vitamin D metabolite concentrations and serum calcium for lead; and in hematocrit, blood cell count, and red blood cell fragility for compounds of arsenic (arsine) and antimony (stibine). Vitamin D metabolite assays utilize sephadex chromatography, high performance liquid chromatography, and competitive binding protein assays.

Gastrointestinal absorption factors for plutonium, and other actinide elements relevant to nuclear power production, are measured in mice, rats, dogs, and baboons. Measurements are made under conditions relevant to setting of drinking water standards. High specific activity isotopes are analyzed in low background facilities by gamma spectrophotometry (^{237}Pu) or by alpha spectrometric-isotope dilution (^{236}Pu, ^{238}Pu).

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Metabolism and toxicity of nonnuclear toxic metals. Changes in gastrointestinal absorption of cadmium and lead during pregnancy and lactation in mice show a striking contrast between the two metals: Cadmium absorption by the dam during pregnancy and lactation increases 2- to 3-fold, with resulting increases in cadmium retention by kidney, liver, mammary tissue, and duodenum. During lactation, less than 8% of cadmium retained by the dam is passed on to pups via milk or excreted via urine. Lead absorption by the dam during pregnancy and lactation increases only 1.4-fold, with little change in lead retention by maternal tissues. During lactation, lead contents of pups and urine both equal lead retained by the dam at sacrifice.

Enovid (norethynodrel:mestranol, 98.5:1.5), when administered orally to mice at a contraceptive dose, produces no changes in the uptake and retention of orally administered ^{109}Cd. The previously observed, 2-fold increase in cadmium retention after oral administration of cadmium during pregnancy in mice was apparently not due to hormonal changes simulated by contraceptives. If our findings can be extended to humans, they indicate that women taking oral contraceptives are probably not at risk from increased accumulation of dietary cadmium.

A system for generating stibine (SbH_3), a toxic gas emitted during charging of lead-acid batteries, has been developed in our laboratory. Although no response was observed in mice exposed to 5 ppm stibine for 60 minutes, hematocrits were two thirds of normal at 5 days after exposure to 50 ppm stibine for 60 minutes, and had returned to normal by 10 days.

Studies on effects of time-of-day of cadmium administration on tissue distribution of cadmium, and on tissue concentrations of the inducible cadmium-binding protein, metallothionein, showed that cadmium deposition in the testis was greatest during the active phase in rats receiving a low intravenous dose of cadmium (48 ng Cd/kg). Metallothionein concentrations in the kidney were highest for rats injected during the active phase with cadmium at a metallothionein-inducing dose (1 mg Cd/kg). (In collaboration with A. L. Cahill and C. F. Ehret.)

Gastrointestinal absorption of actinide elements. Plutonium administered orally in solution adsorbed rapidly to mouse teeth. The resulting problem of determining plutonium content in the carcass after oral administration was eliminated by analyzing carcass-minus-head samples, and applying a correction factor to obtain plutonium content of the entire carcass.

Effects on plutonium absorption of plutonium oxidation state, plutonium hydrolysis, plutonium concentration, animal species, feeding regimen, and age of the animal were identified. Selected results are: (1) Pu(VI) and Pu(IV) are absorbed to a similar extent (0.2%) in the fasted mouse; (2) Pu (VI) absorption is independent of plutonium concentration in the range 10^{-12} M to 10^{-8} M; (3) Pu(VI) absorption by the fed mouse (0.014%) is 10- to 15-fold less than in the fasted mouse (0.19%); (4) retention of absorbed Pu(VI) is 10 to 20 times greater in the fed rat prior to weaning (0.3-0.6%) than in the fed rat at 100 days of age (0.02%). By 7 days after weaning, absorption has decreased to adult levels. Studies with neptunium indicate that Np(VI) absorption in the fasted mouse (0.3%) is similar to that of Pu(VI) (0.2%).

In conclusion, our cadmium studies indicate that exposure to cadmium during pregnancy and lactation may cause increased risk of cadmium toxicity in the mother as a result of increased accumulation of cadmium in her own tissues. Studies to test this possibility are planned for the near future.

Plutonium studies indicate that the value adopted by the International Commission for Radiological Protection for the fraction of soluble plutonium transferred from the gastrointestinal tract to blood in man, 0.01%, is at least an order of magnitude too low.

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13. METAL TOXICANTS: CARCINOGENESIS AND MITIGATION

Y. E. Rahman, P. A. Lagocki, R. A. Schwendener, E. A. Cerny, K. Rogers,* and J. J. Terfruchte+

The objectives of this project are to determine the role of metals in tumor induction, elucidate the mechanisms of their action, and develop new approaches for reducing the tumorigenicity and toxicity of the relevant metal toxicants.

Metal carcinogenesis. (1) Liver system. Metals in various chemical forms are given to mice or rats either by injection or by ingestion, with and without a carcinogen. After different time intervals, the livers of treated and control animals are examined for the appearance of histochemically altered foci and hepatocellular carcinomas. The metals under investigation in these initial studies are iron and arsenic. (2) Skin system. Groups of mice, with and without a toxic metal in their drinking water, and with and without concomitant treatment with the promoter phorbol ester, are treated with either a chemical or a physical carcinogen. Skin lesions are monitored, and tumors are scored morphologically over 8 to 10 months after termination of treatments. Skin lesions are identified histologically after necropsy. Arsenic is the metal under investigation.

Mitigation of toxicity. A new therapeutic approach has been developed for alleviating the toxic effects of metals. This approach involves biological carriers, called liposomes, which are microscopic vesicles made of natural lipids of cell membranes. They are used to encapsulate drugs either in their aqueous compartments or in lipid bilayers. Liposomes varying in size and surface properties are prepared for specific delivery of either a metal chelator or an antitumor drug to target cells. The therapeutic effectiveness of these liposome-encapsulated drugs is then evaluated using appropriate animal models.

A pilot study involving the analysis of preneoplastic foci in the liver in response to administration of iron dextran was carried out. Groups of newborn mice were treated with iron dextran alone or in combination with the liver carcinogen diethylnitrosamine (DEN), followed either by control diet or by diet containing 0.05% of the tumor promoter phenobarbital. Results suggest that (1) a high concentration of iron by itself may act as a complete liver carcinogen for females, but not for males; (2) iron has no promoting activity for animals of either sex; (3) iron, DEN, and phenobarbital

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have a generally additive effect for the production of liver tumors in females, and apparently a synergistic effect in males. To validate these preliminary results with mice, we are carrying out a more extensive experiment using rats, and adding arsenic trioxide in addition to iron dextran.

A pilot experiment for determining the toxic levels of arsenic trioxide, given in drinking water to SKH hairless mice, has been completed. On the basis of the levels determined, we have tested the modifying effect of arsenic trioxide, at different levels, on the induction of skin tumors by ultraviolet radiation (FS40 Sunlamps). Arsenic is known to accumulate in skin, and to inhibit DNA repair. Currently, nine months after the first UV treatment, 36 control mice (receiving only UV and the promoter phorbol ester) show 18 papillomas, 4 carcinomas, and 1 hemangioma; whereas 38 experimental mice (receiving UV, phorbol ester, and arsenic in drinking water at nontoxic concentrations ranging from 2.5 to 20 mg/liter) show 43 papillomas, 10 carcinomas, and 1 possible melanoma. At toxic arsenic levels (40 to 60 mg/liter) no skin tumors have appeared at any time. (In collaboration with Drs. L. S. Lombard and M. J. Peak.)

Using liposomes differing in size and lipid composition, we have studied the characteristics of liposome uptake by the liver parenchymal and Kupffer cells. Large multilamellar liposomes (diameter of about 0.5 μm) were mainly taken up by the Kupffer cells. Unexpectedly, small unilamellar liposomes (diameter of about 0.08 μm) were less effectively taken up by Kupffer cells, and the rate of uptake was slower. The basic mechanism underlying the discrimination of Kupffer cells against small size particles is unknown. In contrast, parenchymal cells were more effective in taking up small liposomes and their uptake of large liposomes was negligible.

Two new water-insoluble iron chelators, namely N,N'-bis[2-hydroxybenzyl]-ethylene-diamine-N,N'-diacetic acid (HBED) and ethylenediamine-N,N'-bis[2-hydroxyphenyl-acetic acid] (EHPG), have been encapsulated in the lipid bilayers of liposomes. The effectiveness of liposome-encapsulated HBED for removing excess iron burden from the reticuloendothelial system of the mouse liver (Kupffer cells) has been compared to that of the most clinically used iron chelator, desferrioxamine (DF), a water soluble drug. Encapsulated HBED is significantly more effective in removing storage iron than liposome-encapsulated DF. On the other hand, EHPG, a chelator with a high specific binding constant for Fe^{3+} similar to that of HBED, is completely ineffective in any form.

Several iron chelators have been chemically modified to obtain optimal incorporation into liposomes. Cholyl hydroxamic acid, stearylamine diethylenetriaminepentaacetic acid (DTPA), and stearylated-DF have been prepared from hydroxamic acid, DTPA, and DF, respectively. A significant increase of liposome incorporation was obtained with the modified chelators: from the usual 10-20% incorporation of the original drugs to 80-100% of the modified forms.

The effectiveness of liposome-encapsulated 1-β-D arabinofuranosylcytosine (araC), given by i.v. or i.p. injection, was tested against Lewis lung carcinoma cells. I.p. injections of liposome-encapsulated araC consistently increased the number of cures (> 120 days survival). These results appear to confirm the finding of Parker et al. (Cancer Research 41, 1311,

1981) which showed that i.p. injection of liposome-encapsulated ^{14}C-Adriamycin, compared with i.v. injection, increases the delivery of the antitumor drug into the lymphatic system where lung tumor metastases occur. Thus, liposomes seem to have delivered araC into the lymphatic system, and to have effectively inhibited tumor growth.

In conclusion, confirmation of our preliminary results suggesting a cocarcinogenic role of arsenic with solar UV irradiation will provide the first animal model for arsenic carcinogenesis.

Further understanding of the discrimination of liver Kupffer cells against small particles will increase our basic knowledge of the phagocytic process and will be useful in applications of targeted drug delivery.

We have demonstrated that liposomes are useful carriers for administration of water-insoluble drugs. In addition, intraperitoneal injection of liposomes containing araC has apparently delivered the drug to the lymphatic system, and has achieved a significant number of long-term cures among mice bearing the highly metastatic Lewis lung carcinoma.

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14. TOXICOLOGY OF COAL GASIFICATION

C. A. Reilly, Jr., F. R. Kirchner, T. Matsushita, M. J. Peak, K. E. Wilzbach,* R. E. Jones, J. M. Leonardo, G. Kaufman, K. T. Blankenship,+ R. M. Bressette,α M. Cannella,** B. Crespi,++ and M. A. Noceraαα

The broad spectrum of potential toxicants present in coal conversion process streams and effluents makes assessment of their impact on human health and the environment a significant task. As one part of an integrated, multidisciplinary Synfuels Environmental Research Program, this project provides toxicologic screening information about coal gasification effluents through a variety of rapid *in vitro* cellular assays and whole animal toxicologic procedures. Present emphasis is placed on the organic by-products of coal gasification (tars and oils). Materials have been studied from three gasifiers: the Institute for Gas Technology's HYGAS pilot plant gasifier (fluidized bed); the Grand Forks Energy Technology Center's pilot plant gasifier (slagging fixed-bed); and Carnegie-Mellon Research Institute's process development unit (coal pyrolysis).

Cellular assay procedures are employed that measure both genotoxicity and cytotoxicity. Induction of bacterial cell mutations are determined using the Ames assay (*Salmonella typhimurium*) and the Mohn test (*Escherichia coli*). Mammalian cell genotoxicity (sister chromatid exchange) and cytotoxicity (growth inhibition and lethality) are evaluated in mouse myeloma cells. Cultured rabbit alveolar macrophages are used to measure subtle functional damage (impairment of phagocytosis) and lethality. A variety of animal toxicologic studies are performed that evaluate the effects of both acute and chronic exposures. Acute exposure effects are determined for whole animals (mouse oral LD_{50}), skin (mouse and rabbit), and eyes (rabbit). Studies of chronic effects are limited to dermal exposure: delayed-type hypersensitivity (albino guinea pig) and carcinogenesis (hairless mouse). Some systemic toxicologic effects are determined in the mice of the skin carcinogenesis study.

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Well over 200 Ames mutagenicity assays from a variety of coal conversion process stream samples and fractions were completed. These assays were performed either for evaluation of sample toxicity or in support of bio-directed chemical analysis. Generally, the less volatile components and tars of coal process streams show the greatest mutagenicity with most of the mutagenicity residing in their basic and neutral components.

The establishment of an Argonne data base for Ames mutagenicity assays has continued. The cumulative spontaneous frequency data base for Salmonella strain TA98 now contains over 300 entries, enabling accurate determinations of mutagenic significance, even with materials of low mutagenicity. The data base for benzo(a)pyrene, used as a positive control, now contains over 100 entries, and a newly established base for 2-aminofluorene contains 50 entries. A new method for analysis of mutagenicity in the Ames assay was developed to supplement the standard measurement of the slope of the dose-response curve. In contrast to the commonly used double background criterion, this computerized method defines the concentration at which a sample becomes significantly mutagenic, based on the cumulative historical spontaneous frequency.

Cytotoxicity and genotoxicity measurements in mouse myeloma cells support the general observations of the Ames test with the exception of the results on water samples. Untreated process waters are significantly toxic for these mammalian cells, even though they are nonmutagenic in the Ames test. Tars and oils were also shown to be moderately toxic as measured by effects on the viability of cultured alveolar macrophages, but these materials were significantly more toxic when cell function (phagocytosis) was used as the end point.

Acute exposure of rabbit skin to oils and tars resulted in "mild to substantial" (National Academy of Science criteria - NAS publication 1138, 1977) inflammatory reactions with some skin necrosis. Rabbit ocular exposures resulted in "substantial to severe" eye irritancy that included inflammatory reactions, corneal ulcers, and pannus, with varying degrees of persistance. Mouse oral LD_{50} test results indicated that these materials can be classified as "slightly toxic." Some materials induced marked skin hypersensitivity while others had no sensitization effect.

In skin studies with SKH hairless mice, HYGAS recycle oil was determined to be a mouse skin carcinogen (weekly 150 µl applications), but the rate of tumorigenic response was less than that for a much smaller dose of benzo(a)-pyrene (0.03 µg/week). The tumorigenic response for similar treatment (105 µg/week) of the nonvolatile components of the recycle oil approximates the response for benzo(a)pyrene. Dermal carcinogenicity experiments with Grand Forks tar are in progress. By 21 weeks of exposure, 80% of the treated mice had tumors. Initiation/promotion studies using Grand Forks tar indicate that these tars may be good initiators.

In summary, while materials have been studied from only a limited number of coal types and process variables (temperature, pressure, etc.), currently available information suggests that there are no uncontrollable toxicologic hazards associated with the coal gasification technologies studied. Some components of coal gasification tars and oils are toxic and carcinogenic, but

in general they are present in the various process streams only in trace amounts and are absent in the end product (substitute natural gas). Nevertheless, control technology and industrial hygiene procedures are warranted to minimize worker exposure to the hazardous materials. The development of correlation methodologies that will increase the predictive powers of the various toxicologic assays performed is an important ongoing activity necessary for more definitive assessment of health impacts.

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S. Bourne, A. M. Jirka, and M. J. Peak
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Chemical and Biological Characterization of High-BTU Gasification (The HYGAS Process) II. Nitrous Acid Treatment for Detection of Mutagenic Primary Aromatic Amines: Non-Specific Reactions
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Isolation and Identification of Mutagenic Primary Aromatic Amines from Synthetic Fuel Materials
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Chemical and Biological Characterization of High-BTU Gasification (The HYGAS Process) IV. Biological Activity
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Bio-Directed Chemical Characterization of Condensates from Synfuel Processes
V. C. Stamoudis, D. A. Haugen, M. J. Peak, and K. E. Wilzbach
Proceedings of the DOE Workshop on Chemical Characterization of Hazardous Substances in Synfuels, Seattle, WA, November 4-6, 1981

15. TOXICOLOGY OF COAL GASIFICATION: CHEMICAL CHARACTERIZATION

D. A. Haugen, M. J. Peak, V. C. Stamoudis,* A. S. Boparai,+ C. A. Reilly, Jr., K. E. Wilzbach,* K. M. Suhrbier, S. S. Dornfeld, D. Venters, and F. J. Tremmel[α]

The objectives of this project are to identify the organic chemicals primarily responsible for the toxicologic activity of synfuel materials, and to investigate their mode of action and biological fate as well as the expression of their toxicologic activity when present in complex mixtures. The long-range objective is to provide information useful (1) for establishing chemical analyses and short-term bioassays to predict toxicologic effects, and (2) for directing possible alterations of process and cleanup conditions to decrease the potential for adverse health effects.

Source of materials. Previously, we examined effluent and process stream samples from the coal gasification pilot plant operated by the Institute of Gas Technology for development of the HYGAS process. We are now examining materials from the slagging fixed-bed gasifier at the Grand Forks Energy Technology Center. For comparative purposes we have also examined reference materials from other synfuel technologies.

Fractionation and Chemical Analysis. Materials are fractionated to isolate classes and subclasses of chemicals. Results of bioassays and chemical analyses after each fractionation step are used as a guide for subfractionation. The chemical composition of mutagenic fractions, as determined by GC and GC/MS, provides general structure-activity relationships, and allows quantitation of the toxicologic activity attributable to reported mutagens.

The major fractionation procedures we have employed at the semipreparative and preparative scales are (1) fractional distillation; (2) liquid/liquid partitioning to separate acidic, basic, and neutral components; (3) silica gel column chromatography to separate neutral components; (4) cation exchange high performance liquid chromatography (HPLC) to separate aromatic bases; and (5) reverse phase HPLC for subfractionation of either basic or neutral components.

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Bioassay. Mutagenic activity of the fractions is assayed according to the Ames assay using Salmonella typhimurium strain TA98 and hepatic S9 from rats treated with Arochlor.

Liquid partitioning demonstrated that (1) the basic fractions (azaarenes and aromatic amines) account for only 2-5% of the sample mass, but about 50% of the mutagenic activity, and (2) the neutral fraction (polycyclic aromatic hydrocarbons and heterocyclics) account for about 70% of the mass and 50% of the mutagenic activity. Mutagenicity has not been detected in the isolated acidic fractions.

A cation exchange HPLC system for fractionation of azaarenes and aromatic amines in the basic fractions has been devised, and used to demonstrate that 3 or 4 ring aromatic amines are the dominant mutagens. We also substantially improved the recovery of 3 to 5 ring aromatic bases in liquid partitioning procedures; bases are extracted from methylene chloride solution using 3.3 N HCl in 43% methanol. Work on the basic fractions has led to plans for study of the effect of alkylation on the mutagenic and carcinogenic activity of aromatic amines.

Work in progress indicates that most of the mutagenic activity in the neutral fractions is present in subfractions containing primarily 2 to 4 ring aromatic hydrocarbons. Some mutagenic neutral components are much more polar than these hydrocarbons, but remain to be identified.

We have extended our study of the inhibition of mutagenesis by complex mixtures such as those obtained by our fractionation procedures. The results show that complex mixtures of aromatic chemicals inhibit mutagenesis by binding to microsomal cytochrome P-450, thus inhibiting monooxygenese-dependent metabolic activation. We have been unable to attribute the inhibition to any specific subclasses of chemicals. Thus, our results indicate that mutagenesis assays of complex mixtures must necessarily be considered less quantitative than results from assays of individual components.

In summary, we have devised and improved methods for isolation of aromatic bases and have demonstrated that, for each sample examined, a relatively small number of 3 or 4 ring aromatic amines are responsible for most of the mutagenicity in each of the basic fractions. Progress has been made in isolation of the nonpolar components principally responsible for the mutagenic activity of neutral fractions. We have provided a better understanding of the inhibition of mutagenic activity occurring for complex mixtures of polycylic aromatic chemicals.

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16. NEUROBEHAVIORAL CHRONOBIOLOGY AND TOXICOLOGY

C. F. Ehret, A. L. Cahill, R. S. Rosenberg, P. H. Duffy, K. R. Groh, J. J. Russell, J. V. Barnett,* A. S. Gulczynski,+ R. Scott,α and G. H. Thomsen**

This new program is concerned with the measurement of neurotoxicological damage caused by energy related agents and conditions. With a common focus on changes in established biological rhythms, the program integrates physiological, neurochemical, and behavioral studies. The program represents a consolidation and reorientation of our previous studies, reported below, which have been in two program areas.

Chronobiology and Circadian Regulation

The goal of this program has been to elucidate the molecular events underlying circadian regulatory processes in mammalian systems. Emphasis was placed on the actions of diet, drugs, and environmental agents upon circadian phase, and on the coupling of such actions with neurochemical function. Recent studies have focused on the role of inhibitors of catecholmine synthesis in circadian metabolism in the brain, and on the role of meal timing and of lighting regimens in causing dyschronism. Automated and programmable environmental chambers were used for entrainment by meal timing and light-dark cycles, and for recording food consumption and deep-body temperature in Charles River rats.

The catecholamine synthesis inhibitor α-methyl-p-tyrosine (AMT) is chronobiotically active. Since, as we have shown, turnover rates of norepinephrine and dopamine vary as a function of the phase of the circadian cycle, it might be expected that the physiological effects of inhibiting tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of catecholamines) with AMT at various times throughout the day would be different. In fact, this has been observed: AMT shifts the acrophase (time of highest temperature) of the circadian temperature rhythm of the rat to earlier or later times of day depending upon the phase of the circadian cycle at which the drug is administered.

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These times are correlated with the times of minimal and maximal turnover of norepinephrine in the hypothalamus. This observed chronobiotic action of AMT is consistent with other evidence for a central role of catecholamines in the regulation of circadian rhythms. The results also emphasize the fact that the effects of drugs may vary as a function of the time of day. This fact must be taken into account in clinical medicine and in pharmacological testing.

Meal-timing and selection of rotation schedules for shift work ameliorate the dyschronogenic action of simulated shift work rotation schedules in rats. In animal studies that modeled four of the most commonly used shift work rotation schedules (ROTAS), significant differences were seen among the ROTAS in the ability of the animals to adjust their circadian body temperature rhythms to the shifts. The ROTAS investigated were: (1) slow rotation of "shifts" by a weekly 8-hour phase delay, (2) slow rotation by a weekly 8-hour phase advance, (3) rapid rotation in which phase delays of 8 hours occurred daily, and (4) rapid rotation in which an 8-hour phase delay was introduced every other day ("2-day"). The experiments showed that slow rotation by phase delay with anticipatory meal timing on "days off" resulted in the most rapid phase adaptation to the new shift with minimum disturbance of the normal circadian temperature rhythm. The other protocols were dyschronogenic, especially the two rapid rotation schedules, and, of these, "2-day" rotation was worse than the daily rotation. These findings have significant consequences for the widely used metropolitan and continental shift schedules, which are based on the "2-day" scheme.

Effects of 60-Hz Electric Fields on Ultradian and Circadian Rhythms of Physiological and Behavioral Functions

Our objectives were to investigate the effects of 60-Hz electric fields on the ultradian (less than a day) and circadian rhythms of physiological and behavioral end points in rats and mice. Brief exposures above 35 kV/m, presented during the inactive phase of the circadian cycle, produce an arousal response in both mice and rats characterized by increased in motor activity, CO_2 evolution, and O_2 consumption. To test the hypothesis that vibration of hair and vibrissae induced by exposure is a primary mechanism for arousal, we used the SKH:hr-1 hairless mouse, removing the few vestigial vibrissae. Persistence of arousals in hairless mice showed that stimulation of hair and vibrissae cannot be the sole mechanism for the arousal response induced by electric fields.

We have also found that brief exposures above 100 kV/m presented to Peromyscus leucopus mice during the early active phase of the circadian cycle cause significant circadian phase delays in activity and metabolism acrophases. "Splitting" of activity into two distinct components, and circadian episodes of torpor were also observed in exposed groups. Our new findings clearly show that 60-Hz electric fields can act as circadian zeitgebers.

In conclusion, our earlier studies and those reported here have shown the power and utility of the methods of ultradian and circadian waveform analysis in defining previously unsuspected actions of environmental agents on organisms. Traditional methods of assay for threshold limit values of potentially toxic agents and conditions have been confined in the past to use of mutagenic,

carcinogenic, teratogenic, and lethal actions as end points. Our chronobiologically oriented methods ask instead whether an agent or condition is dyschronogenic. Chronobiotically active drugs, multiple zeitgebers out of phase, and high voltage electric fields are all potentially dyschronogenic agents and conditions. Our new program will focus on an understanding of the normal ultradian and circadian oscillations of physiological, neurochemical, and behavioral variables, with particular emphasis on long-term in vivo monitoring of neurotransmitter levels and of sleep-wake patterns. These chronobiological methods are highly relevant to the previously neglected area of the etiology of behavioral and psychological disorders, an area now closely associated symptomatically with circadian dyschronism and with marginally disturbed circadian and ultradian waveforms.

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Glucocorticosteroid Injection is a Circadian Zeitgeber in the Laboratory Rat
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17. BIOSTATISTICS AND HEALTH IMPACTS OF ENERGY TECHNOLOGIES

M. E. Ginevan, B. A. Carnes, J. J. Collins, C. D. Brown, J. R. B. Curtiss, N. Devine, and A. Rock*

The present research develops statistical methodology and mathematical models relevant to the assessment of health impacts of energy technologies. Efforts have included: (1) Development of a statistical procedure for reducing dimensionality in studies of disease-environment association. (2) Development of a maximum eigenvalue least squares (MELS) theory for identifying, quantifying, and circumventing collinearity in regression models. (3) Evaluation of statistical model violations in an analysis of covariance (ANCOVA) study of air pollution. (4) Development of an age-period-cohort model of breast cancer.

The ultimate goal of these related lines of inquiry is to provide a unified body of fact and theory for data analysts responsible for the assessment of health impacts. To this end, the following methods were employed:

1) Subsets of predictor variables associated with general factors (e.g., socioeconomic, demographic, pollution) are combined by ordinary least squares (OLS) regression weights to construct a reduced number of synthetic variables in a regression equation.

2) Principal components analysis (PCA) conducted on the correlation matrix of predictor variables identifies and quantifies collinearity problems. Eigenvectors associated with collinearity are dropped from the analysis using a rule based on the condition of the correlation matrix. The remaining eigenvectors are used to generate a MELS regression solution.

3) Homogeneity of groups defined by dummy coded variables in an ANCOVA is evaluated by multiple group discriminant analysis. If the assumption of homogeneity is rejected, a PCA clustering procedure is used to identify homogeneous assemblages. A general linear model testing procedure is then used to arrive at an acceptable model.

4) The application of the age-period-cohort model required construction of a set of data by age, sex, and year for the incidence and mortality in the United States between 1900 and 1975 for female breast cancer. Weighted least squares (WLS; restricting cohort parameters to zero) and MELS solutions (dropping near zero eigenvectors) are generated and compared.

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From these methods, the following results were obtained:

1) The R^2 using synthetic variables is identical to the R^2 using the full set of predictor variables, indicating that the predictive power of the model has not been distorted. Dimensionality is greatly reduced by the use of synthetic variables. The synthetic variables, representing general categories, provide a more realistic rendition of the original hypothesis that initiated the analysis and led to the selection of variables for the regression model.

2) Eigenvectors associated with zero or near zero eigenvalues identify linear dependencies (collinearity) in the data structure. Dropping these eigenvectors from the analysis reduces the variance of the resulting regression estimates at the cost of introducing some bias. In the case of perfect collinearity (singularity), this procedure provides a solution (where none exists for OLS) that is identical to a Moore-Penrose generalized inverse solution (e.g., age-period-cohort mortality models).

3) The homogeneity of the classification scheme based on census regions was rejected by discriminant analysis. Therefore, using dummy coded variables for census regions in an ANCOVA is inappropriate. Failure to consider the general linear test for parallelism results in significant pollution effects in the ANCOVA model of mortality. However, separate regressions conducted within homogeneous assemblages (of U. S. counties generated by PCA clustering) necessitated by the rejection of parallelism in our analysis results in nonsignificant air pollution effects. This analysis demonstrated the severe distortions of regression estimates and, hence, interpretation when the assumptions of a statistical model are violated.

4) The age-period-cohort approach is useful for determining general trends and isolating time-specific influences. In breast cancer, little or no decline in mortality results from medical intervention, and most changes in trend from this disease appear to be related to the fertility patterns of the population. The WLS solution based on historical evidence and the MELS solution yield very similar results and provide support for the constraints imposed by the two approaches.

In addition to the above studies, the following investigations, supported by the Nuclear Regulatory Commission, have been essentially completed: (1) a dose-response and demographic model useful in the prediction of the health effects of energy technologies; and (2) a reanalysis of the Tri-State leukemia survey data, focusing on the relationship between myelogenous leukemia risk and diagnostic X-ray exposure.

In conclusion, the general linear model (regression, analysis of variance, analysis of covariance) is the principal tool of the applied data analyst. Therefore, our goal has been to develop a framework of statistical strategies involving the general linear model that minimizes the likelihood of assumption violations. This protection is especially important when results from such analyses are used in the assessment of health impacts. Future efforts will be directed toward expanding our basic research in applied statistics. Specifically, we will evaluate the robustness of the MELS solution to

extreme values (outliers) and the sensitivity of R^2 in MELS solutions when the response variable is associated with minor dimensions of the data structure.

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THE HUMAN PROTEIN INDEX

18. THE HUMAN PROTEIN INDEX

N. G. Anderson, N. L. Anderson, J. J. Edwards, C. S. Giometti, J. Taylor, K. E. Willard, M. A. Gemmell, S. L. Nance, A. E. Scandora,* S. L. Tollaksen, B. Mallard,+ and M. T. Powers[α]

Fundamental to this program is the use of two-dimensional electrophoresis to establish an annotated index of human proteins which will include data on normal proteins, genetic variants, and disease-related changes. The establishment of such a human protein index requires the development of methods for running large numbers of analyses in a reproducible manner, the development of computerized image analysis and data base systems, and the production of protein maps of major body fluids and accessible cells and tissues.

It is evident that the development of methods for "seeing" large numbers of human proteins analytically, for detecting compositional changes, and for ultimately identifying or assigning function to as many proteins as possible is basic to a broad spectrum of biological research. For example, the methods are applicable to studies of the effects of radiation and energy related pollutants on man and on human cells, on mutational events, on molecular alterations related to carcinogenesis, on the identification of proteins that bind or transport toxic agents, and on the clinical detection of injury.

Most disease and toxic injury ultimately expresses itself in alterations in the structure, amount, or cell location of proteins, or in the time of their expression during human development. Thus, mutations may produce proteins that have an altered amino acid composition, which in turn may grossly affect cell function, resulting in secondary compositional or structural changes. Toxic injury may alter cell structures, especially membranes, producing a redistribution of internal components and, in many instances, leakage of protein from the cell. In addition, toxic agents may bind to specific proteins and be transported by them (for example in milk), or they may disturb the function of the bound protein. Human cells contain large numbers of proteins; estimates of the number coded for by the human genome range from as low as 10,000 to over 100,000, with most estimates falling between 30,000 and 50,000. Only a few percent of these proteins have been identified as to function or cell type in which expressed, and an even smaller fraction have been analyzed in detail.

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[α]Abbott Laboratories.

The only technique presently available for the development of the human protein index is high-resolution two-dimensional electrophoresis. Based on the ISO-DALT systems we have developed for doing multiple parallel two-dimensional electrophoretic analyses, methods and procedures for indexing protein gene products have been worked out that list positional coordinates with reference to standards and available analytical data. Each spot has a serial number, as well as one or more common names, and represents a unique protein having a variety of determinable properties. Three types of characteristics of proteins on the two-dimensional gels can be obtained: (1) location in the cell, (2) physicochemical properties (approximate amino acid composition, phosphate content, thermostability, SH content, interaction with small molecules), and (3) regulatory behavior (regulation by effectors, alteration during differentiation, etc.).

Computerized data reduction methods are used that enable rapid noise subtraction, background flattening, resolution of spots into two-dimensional Gaussian curves, normalization of a spot pattern with reference to a standard, and the preparation of spot lists. The software developed is capable of fitting five parameter two-dimensional Gaussians to 1200 spots simultaneously.

With the development of operational systems, we have turned our attention to the acquisition of the basic data required before meaningful studies on human populations (normal, diagnosed as ill, exposed). We have published standard two-dimensional maps of the proteins of human plasma, muscle, lymphocytes of various types, red cell lysates, seminal plasma, saliva, and urine. Over twenty protein sets in human lymphocytes have been discovered that are affected by chemical agents, including phorbol esters, interferon, calcium ionophores, and antimitochondrial drugs. To review the diagnostic significance of this and parallel work, a joint Mayo Clinic-Argonne symposium was held in November of 1981.

In summary, we consider that the feasibility studies for the human protein index are complete and that work on the compilation of data for the index can be pushed forward.

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D. S. Young and N. G. Anderson
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B I O P H Y S I C S

19. PROPERTIES OF BIOLOGICAL MOLECULES IN SOLUTION

M. MacCoss, H. M. Schwartz, C. F. Ainsworth, S. H. Gray,* M. McCroskey,+ and R. J. Ross[α]

This project has as its principal goals (1) the synthesis (or isolation) and chemical characterization of naturally occurring biomolecules and specifically modified analogues; (2) determination of quantitative structural/conformational parameters for those molecules; (3) correlation of structural and conformational properties with biological function.

A full repertoire of organic chemical synthetic methods is employed, and in many cases new synthetic techniques and methodologies are developed in order to prepare the desired compounds. Since a heavy emphasis is placed on purity of products, chromatographic and electrophoretic techniques are exploited fully to separate complex mixtures. All new compounds are fully characterized by chromatography, elemental analysis, melting point, and spectroscopic (NMR, UV, MS, and IR) measurements.

The structural aspects of this work rely on high resolution NMR techniques and small-angle X-ray and neutron scattering methods (see Section 21).

Continuing our endeavors in the field of the ^{17}O NMR of nucleic acid components, we have extended our synthetic work to the preparation of ^{17}O-labeled uridine derivatives bearing a variety of substituents, with different electronegativities, at the 5-position of the heterocyclic ring. Such derivatives, namely, [2-^{17}O]- and [4-^{17}O]-5-fluorouridine, [2-^{17}O]- and [4-^{17}O]-5-bromouridine, and [2-^{17}O]- and [4-^{17}O]-5-methyluridine (ribothymidine) have all been prepared and examined by ^{17}O NMR spectroscopy in order to evaluate the changes induced in the π-bond order at the C2 and C4 carbonyl functions, and to relate these to hydrogen-bonding changes at these positions due to electronic perturbations in the heterocyclic base ring.

Our investigations on nucleoside N-oxides (including a new synthesis of 2-azaadenosine N^1-oxide) have led us to a new hydrolytic pathway for 2-azaadenosine N^1-oxide. The first major isolatable breakdown product in basic solution has been shown to be 5-azido-1-β-D-ribofuranosylimidazole-4-carbonitrile [presumably via the 5-(3-hydroxytriazene)-1-β-D-ribofuranosylimidazole-4-carbonitrile]. This azido derivative shows unexpected

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+Spring 1981 participant in the Undergraduate Honors Program, Maryville College, Maryville, TN.

αParticipant in the 1981 Summer Biology Research Institute, Northern Illinois University.

lability under hydrolytic conditions and degrades to several products. The mechanism described above was investigated by ^{1}H NMR and verified by the use of chemically synthesized [2-^{15}N]-2-azaadenosine-N^{1}-oxide and ^{15}N NMR spectroscopy.

^{1}H NMR studies on the eight dXparaC and dXparaA (where X = T, A, C, or G) deoxynucleosidyl-3' → 5'-arabinosides are now nearing completion, and have indicated markedly different conformational characteristics relative to the unmodified dXpdC and dXpdA derivatives. In addition to helping to understand the forces involved in fixing the conformational properties of nucleic acid tracts, this work is providing a solid structural basis for the inhibition of DNA polymerase, and thus for the precise nature of the cytotoxicity related to arabinonucleosides.

In our continuing studies of nucleoside-phospholipid conjugates, we have developed a general procedure for synthesis of the precursor L-α-phosphatidic acids starting from the respective L-α-phosphatidylcholines using phospholipase D. This method reduces the cost of preparation tenfold and has enabled us to prepare araCDP-L-dipalmitin, araCDP-L-dimyristin, araCDP-L-distearin, araCDP-L-diolein, and the radioactive derivative araCDP-L-di[1-^{14}C]palmitin in good yield as analytically pure samples. The derivatives have been investigated for their biological efficacy (in collaboration with C. I. Hong, Roswell Park Memorial Institute and T. Matsushita) and their aggregational and morphological characteristics (collaboration with J. J. Edwards and T. M. Seed of this division, and S. P. Spragg, University of Birmingham, U. K.). Gel-filtration, viscosity measurements, and electron microscopy studies have shown the nucleoside-phospholipid conjugates to undergo unusual morphological transitions (from bilayer sheets to a three-dimensional hydrated gel) at the phase transition temperature. In addition, other morphological forms (small micellar discs) can be produced by sonication close to the transition temperature. The extent to which the morphological structure is related to the biological efficacy is, of course, an important consideration under investigation.

In summary, work is continuing on the conformational properties of key biological molecules in solution, using a variety of biophysical techniques. Central to the program is the use of chemical synthetic methods or biochemical isolation techniques to obtain the molecules under investigation. In addition, a constant attempt is made to utilize or devise new procedures to these ends. Major interests center around the use of NMR and other biophysical techniques to evaluate structural and topographical parameters of the molecules under investigation. In the future, greater emphasis will be placed on developing synthetic methods to augment the small-angle X-ray and neutron scattering work being carried out in this group (i.e., development of the use of bifunctional chelating agents for attachment of metal ions to specific amino acid residues in proteins). (See the following sections.)

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Coordination and Binding of Taurine as Determined by Nuclear Magnetic Resonance Measurements on ^{13}C-Labeled Taurine
C. S. Irving, B. E. Hammer, S. S. Danyluk, and P. D. Klein
Workshop on Taurine: Questions and Answers, Mexico City, Mexico, November 16-19, 1980

20. STRUCTURAL STUDIES OF IMMUNOGLOBULINS

M. Schiffer, M. T. Short, F. J. Stevens, and F. A. Westholm

The overall goal of this project is to understand the function and specificity of antibodies on the molecular level. X-ray diffraction techniques are used to determine the precise three-dimensional structure of immunoglobulins in the crystalline state. The results are complemented by data obtained from gel filtration and small-angle neutron and X-ray scattering studies of antibody molecules in dilute soltion. So far, emphasis has been placed on structure determination of dimers formed by imunoglobulin light chains, Bence-Jones proteins, which form excellent models of intact antibody molecules. To examine the effect of antigen-antibody interaction on the structure of the antibody binding site, work has been initiated on functional antibodies against protein antigens. We are studying rheumatoid factors (autoantibodies against IgG proteins) and antibodies against immunoglobulins prepared by hybridoma techniques.

Proteins are purified by column chromatography; crystallization is performed both by batch and vapor diffusion methods with ammonium sulfate or polyethylene glycol. Data on single crystals are collected by a computer-controlled diffractometer, and new crystals are characterized by precession techniques using a high intensity X-ray beam from a rotating anode X-ray generator. X-ray and small-angle neutron scattering techniques are applied. Extensive use is made of the excellent Argonne central computing facilities and services.

The structure of the Mcg Bence-Jones dimer ($\lambda\gamma$-type light chain) has been determined and the crystallographic refinement with 2.3 Å resolution data is in progress. The radius of gyration of the Mcg Bence-Jones protein was measured by small-angle neutron scattering at Brookhaven National Laboratory. The radius of gyration calculated from the refined crystallographic coordinates shows that the Bence-Jones protein has a similar structure in solution to the structure observed in its crystalline form.

About 50 different Bence-Jones proteins have been characterized and purified; Dr. A. Solomon (University of Tennessee) has characterized these proteins clinically and immunochemically. Our attempts to crystallize both the mono-

meric and dimeric forms have yielded microcrystals for eight of these proteins. Further, we were successful in growing crystals in forms suitable for X-ray diffraction studies from κ_{II}-type protein Fin, λ_I-type proteins Cle and Loc; and from the variable fragment derived of κ_I-type protein Wat.

We have characterized crystals grown from a new λ_I Bence-Jones protein, Loc, and determined its structure to 5Å resolution using the method of multiple isomophous substitution. Protein Loc has been shown to consist of two covalently bound intact chains, and its amino acid sequence is being determined by Dr. H. Deutch (University of Wisconsin). Crystals suitable for X-ray diffraction measurements were obtained using one of three different precipitants, ammonium sulfate, polyethylene glycol, and distilled water. All of the crystals are orthorhombic $P2_12_12_1$, but the cell dimensions of those grown in ammonium sulfate ($\underline{a}$ = 149.3, $\underline{b}$ = 72.4, and $\underline{c}$ = 46.5 Å) differ from the dimensions of the crystals grown in the other two solvents ($\underline{a}$ = 119.0, $\underline{b}$ = 74.2, and $\underline{c}$ = 50.5 Å). Diffraction data for the native crystal grown from ammonium sulfate were collected to 3.5 Å resolution. Twelve different data sets of heavy atom derivatives were collected to 5 Å resolution. Using difference Patterson and difference Fourier techniques, the heavy atom positions were found for the $K_2Pt(CNS)_6$, o-chloromercuriphenol derivatives, and the covalent Hg derivative, where Hg was inserted into the interchain disulfide bond. An electron density map, calculated using data to 5 Å resolution, showed distinguishable boundaries of protein and solvent. Further, we were able to identify the four domains of the molecule and the approximate locations of the local twofold axis between pairs of variable and constant domains. The elbow bend is less than that of the Mcg protein.

New data on larger crystals were collected for the κ_I Wat protein variable segment. Preliminary results of the molecular replacement studies suggest that in this protein the domain-domain interactions are different from those observed for other variable fragment crystals.

A major improvement in our data collection was accomplished by the recently installed diffractometer control system. We have further developed the system to simplify alignment procedures and have set up a new mode of data collection that gives more accurate intensity values for plate-like crystals.

In summary, the structure of a λ_I-type light chain dimer, protein Loc, has been determined at 5 Å resolution. It will be possible to determine the structure of this protein to atomic resolution, since the crystals diffract to 2.3 Å. The determination of the structures of different classes of light chains (such as protein Loc) will further our knowledge of the antigen binding site, and of the antigenic and idiotypic determinants of the light chains. We are continuing our efforts to crystallize functional antibodies against protein antigens.

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J. Mol. Biol. 147, 179-183 (1981)

In Press

Small-Angle Neutron Scattering Study of Bence-Jones Protein Mcg: Comparison of Structures in Solution and in Crystal
M. Schiffer, F. J. Stevens, F. A. Westholm, S. S. Kim, and R. D. Carlson
Biochemistry

21. NEUTRON AND X-RAY SMALL-ANGLE SCATTERING

C. S. Borso, B. I. Berkoff,* and C. T. Mizumoto+

The principal objectives of this project are to develop unique X-ray and neutron small-angle scattering facilities and to utilize them to study the structure and dynamics of individual biological molecules and complexes of interacting macromolecules. New instrumentation is being developed to provide fundamentally new biophysical techniques for structural investigations of important biological molecules, with particular emphasis on calcium-dependent proteins involved in cellular regulation. Major emphasis is placed upon the development of techniques to utilize important new facilities for X-ray and neutron production to take advantage of their unique characteristics. Time-of-flight diffraction techniques are being developed to utilize resonance small-angle scattering at spallation pulsed neutron sources, and novel detector technology is being employed to increase the temporal resolution available from high intensity synchrotron sources to study the structural dynamics of proteins.

A novel self-scanning photodiode array detector which we have developed is utilized routinely for X-ray small-angle scattering studies of proteins in solution. We have developed computer hardware and software that are used to determine values for various physical and structural parameters such as the radius of gyration, the extrapolated forward scattering, cross-sectional radius of gyration, cross-sectional mass per unit length, volume, average electron density, and intramolecular electron density distribution.

Time-of-flight (TOF) diffraction techniques have also been developed for utilization at the Argonne pulsed neutron source, IPNS-I. Data acquisition software has been implemented by F. S. Williamson of this division to allow us to extract many of these same small-angle scattering parameters from neutron data. An automated sample changer installed on the TOF instrument facilitates measurements of varying neutron scattering length densities in solutions. This method is essential to easily contrast match regions of similar scattering density to differentiate lipids, proteins, nucleic acids, and synthetically labeled moieties from other subunits of the macromolecule under study. Small-angle neutron resonance scattering techniques are being developed to provide new biophysical methods of structural determination in biological molecules.

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*Summer 1981 participant in the Undergraduate Honors Program, Princeton University.

+Fall 1981 participant in the Undergraduate Honors Program, Pacific Union College.

X-ray small-angle scattering. Solution studies of detergent-solubilized bovine rhodopsin from the outer segment disk membranes of rod cells from the retina, conducted in collaboration with Dr. M. Applebury, Princeton University, have provided interesting results on this important protein. We have measured the radius of gyration of the solubilized protein and its associated lipid micelles in solution at various solvent electron density levels. Our results provide clear evidence that other investigators have been using rhodopsin dimers for structural studies and that reported elongation of this possibly transmembranous molecule is not observed, at least in solution.

A series of small-angle experiments has been undertaken to study oxygenated and deoxygenated normal and sickle-cell hemoglobin to understand the structural basis of prefiber gelation of deoxygenated sickle hemoglobin. Measurements are being made as a function of concentration and temperature to characterize the intermediate aggregates with the ultimate goal of studying drug interactions designed to inhibit prefiber aggregation. (In collaboration with Drs. M. Johnson and P. Thiyagarajan, University of Illinois Medical Center.)

The calcium binding protein, calmodulin, has been studied in small-angle scattering experiments in both metalized and demetalized form. The results indicate that a considerable structural transformation takes place upon the binding of four Ca^{2+} ions to increase the radius of gyration value of calmodulin by 3 Å when the molecule is metalized. In addition to observing an increase in size instead of a decrease, the magnitude of the observed change was quite unexpected and is under further investigation.

Changes in the cross-sectional radius of gyration and effective mass per unit length of segments of DNA caused by counter ions in solution have been studied with small-angle scattering. Large effects have been observed with different counterions in an attempt to perform the first structural investigation to probe implications of the Manning theory of polyelectrolytes in biological solutions.

Pulsed neutron small-angle scattering. TOF data from scattering experiments at the IPNS-I pulsed spallation source have been analyzed on several solutions of biological interest. Cetylpyridinium chloride in H_2O/D_2O solution mixtures has been utilized to calibrate the new instrument and data acquisition system. The first small-angle scattering from various proteins has also been observed using samples of cytochrome c, hemoglobin, and canine high density lipoprotein. These different samples were utilized to obtain scattering over a range of values of momentum transfer at different wavelengths and with various components of incoherent and parasitic background scattering. Data analysis is incomplete, but preliminary results suggest that a reasonable value for the radii of gyration of these proteins will be obtained. The TOF small-angle instrument has been developed in collaboration with the Material Science Division and similar software has been written in this division to analyze the small-angle scattering from strongly scattering metallurgical samples. We are confident that the weaker biological scattering already observed can be analyzed to extract the relevant small-angle scattering parameters.

In summary, both X-ray and neutron small-angle scattering facilities are being developed to study the solution structures and dynamics of biological molecules. The techniques made available with these facilities can be utilized to provide complementary information, and both methods have been sufficiently developed to provide fundamental structural information on molecules of interest. Our attention will be focused on the application of these small-angle scattering techniques to structural problems relevant to calcium binding and calcium-mediated regulatory processes.

PUBLICATIONS, 1981 THROUGH APRIL 1982

In Press

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C. S. Borso, J. M. Carpenter, F. S. Williamson, G. L. Holmblad, M. H. Mueller, J. Faber, Jr., J. E. Epperson, and S. S. Danyluk
J. Applied Crystallography

APPENDICES

22. SUPPORT FACILITIES

ANIMAL FACILITIES

T. E. Fritz, J. M. Angerman, W. G. Keenan, L. S. Lombard, C. M. Poole, A. R. Sallese, and D. V. Tolle

The Laboratory Animal Facilities are a major resource of the Division and occupy approximately 34% or 40,000 square feet of the space in the Biology Building. They consist of four major components: (1) dog kennels with a capacity for approximately 700 dogs; (2) two building wings, Wings E and Q, consisting of 47 animal rooms which are capable of housing small laboratory animal species; (3) service areas which include equipment for cleaning and sanitizing cages, water bottles, and cage racks, as well as storage space for food and equipment; and (4) technical equipment areas which include examination and procedure rooms, a surgical operating room, necropsy room, diagnostic X-ray rooms, and a "hot room" for handling radioactive materials.

In addition, three of the four Divisional ^{60}Co gamma ray exposure facilities are physically related to the animal facilities and are dedicated to whole-body irradiation studies in animals.

During 1981, approximately 16 staff members of the Division were involved in various studies utilizing in excess of 19,000 rodents and other laboratory animal species.

The supporting facilities for the husbandry and clinical monitoring of the resident animals and the responsible personnel include:

1) Automated cage and bottle washing and filling machinery (Animal Care Specialists)

2) Steam and gas autoclaves for sterilization of equipment, instruments, and media (Animal Care Specialists and scientific staff)

3) Diagnostic X-ray facilities and darkrooms (C. M. Poole, W. G. Keenan)

4) Clinical pathology laboratory (D. V. Tolle)

5) Hematology laboratory (D. V. Tolle)

6) Necropsy laboratory (T. E. Fritz)

7) Histopathology laboratory (T. E. Fritz, L. S. Lombard, A. R. Sallese)

8) Surgical suite with inhalation anesthesiology equipment (C. M. Poole, W. G. Keenan)

An important aspect of the success of any animal research program is the health of its animal population. To assure a supply of high quality animals, the Facility has concentrated on breeding its own healthy, disease-free animals. Most of the rodents, particularly mice, and all dogs are bred in the Facility. The rodent breeding is managed by J. M. Angerman. The beagle breeding is supervised by C. M. Poole and W. G. Keenan, who are also responsible for the clinical and surgical care of the dogs. The beagle colony has been a closed colony for more than 20 years, and extensive computerized records are maintained under the supervision of D. E. Doyle on all aspects of the colony, including reproduction, genetics, hematology, pathology, and disease incidence.

As important as the physical plant and its support facilities is the staff available to manage, monitor, treat, and evaluate the animals. The personnel provide a complete range of services to users of the experimental animals. Care of the animals is performed by a group of Animal Care Specialists, listed below, under the supervision of E. W. Jackson.

The Animal Care Specialists during 1981 were the following:

Claude C. Colegrove, Group Leader
William G. McDade, Jr., Group Leader
Adrian J. Cordova
Mose Burrell
Lucille E. Daley
Charles J. Fowler
MaDonna Gandalovics
Rita Girard
Carrey R. Herringer
Robert Herringer
Cathleen L. Higgins
Betty L. McKay
William O. Robinette
Anthony M. Rodriguez
Bernard A. Royer
Richard M. Santarelli
Rika Simmons
Leon L. Stewart
Diane M. Thomas
Jose Torres
Joseph N. Wilson

RADIATION FACILITIES

G. L. Holmblad, J. L. Hulesch, J. E. Trier, and F. S. Williamson

The Divisional radiation facilities include a number of gamma, neutron, and X-ray radiation sources with accompanying areas for related equipment and preparing, handling, and servicing animals. These sources are described in detail below. There are five ^{60}Co irradiation facilities which provide a unique and versatile gamma-ray irradiation capability. A research reactor (JANUS) is a source of fission-spectrum neutrons dedicated to biological research. The gamma-ray facilities are suitable for long-term studies in which animals can be maintained in the radiation field for duration of life or for acute brief or fractionated exposures. They provide a choice of exposure rates ranging from 0.004 to 2 x 10^4 R/minute. The mean energy of the JANUS neutrons is 0.85 MeV, and the available dose rates range between 0.002 to 80 rads/minute. The contamination from gamma radiation is remarkably low, between 3 and 4 percent of the total absorbed dose. X-ray facilities consist of two X-ray machines, one for experimental irradiations and one for clinical diagnostic purposes. Research using the Divisional radiation facilities is described principally in Sections 2, 3, and 4 of this report.

In addition to the radiation sources maintained in the Division for the irradiation of biological materials, two other neutron sources in the Chicago area are available to the staff for cell biology studies comparing neutron beams with different characteristics: neutrons from the linear accelerator at the Cancer Therapy Facility of the Fermi National Accelerator Laboratory ($\underline{p}^+ \rightarrow$ Be, mean energy 25 MeV) and cyclotron produced neutrons ($\underline{d}^+ \rightarrow$ Be, mean energy 3.6 MeV) at the Franklin McLean Research Institute, The University of Chicago.

^{60}Co Gamma Radiation

F-005 Gamma Room Radiation Field Dosimetry

Cavity configuration. Exposure rates from 500 to 16,000 R/minute with ± 2% variation within volumes as large as 25 cubic centimeters.

Panoramic configuration. Exposure rates from 20 mR/minute to 8 R/minute with a worst case deviation of 7% from the mean within an arc large enough to contain 500 small rodents. Exposure rates as high as 30 R/minute can be achieved with up to 80 animals.

F-101 Gamma Room Radiation Field Dosimetry

Panoramic configuration. Exposure rates from 1 mR/minute to 30 mR/minute by varying distance and attenuation. Up to 500 small rodents may be irradiated simultaneously at four different expo-

sure rates with a worst case deviation of 7% from the mean. Animals are maintained in individual plastic cages, with food and water, on a frame with an N x 5 matrix. N varies from 4 to 8 depending on the frame distance from the source.

F-114 Gamma Room (Intermediate) Radiation Field Dosimetry

Panoramic Configuration. Exposure rates from 5 R/day to 40 R/day by varying distance and attentuation. Up to 52 dogs may be irradiated simultaneously at four different exposure rates.

X and Y Gamma Rooms Radiation Field Dosimetry

Panoramic configuration. In room X, exposure rates of 1.0 R/day and 2.5 R/day on two arcs, each of which can contain up to 50 individually caged dogs. In room Y, exposure rate of 0.4 R/day on one arc which can contain up to 100 individually caged dogs.

Fission-Spectrum Neutron Radiation

JANUS Reactor Radiation Field Dosimetry

Panoramic configuration. Dose rates from 0.002 to 15 rads/minute over a matrix of 400 mice with a worst case deviation of 8% from the mean. The gamma dose contribution is only 3-4% of the total absorbed dose.

Cavity configuration. A restricted room area near the converter plate may be used for irradiating small samples of cells at up to 65 rads/minute with good uniformity.

X-Radiation

General Electric Maxitron 250-kV X-ray machine, a general Divisional facility.

Westinghouse Flurodex "300" 130-kV X-ray unit equipped with a Machlett, Dynamax-40, rotating anode, dual focal spot tube. Used for clinical and diagnostic purposes for small animals and dogs.

23. EDUCATIONAL ACTIVITIES

POSTGRADUATE TRAINING

During 1981, a total of 23 postdoctoral appointees and research associates contributed to the research programs of the Division. Five of these were new appointments in 1981, while nine finished their assignments during the year.

The temporary appointees, their schools, and the staff members with whom they were affiliated were as follows:

Franco M. Buonaguro	Instituto di Patologia Generale, Naples, Italy	M. M. Elkind
Anne L. Cahill	University of Michigan, Ann Arbor	C. F. Ehret
Bruce A. Carnes	University of Kansas	M. E. Ginevan
Pamela L. Derstine	Northwestern University	T. Matsushita
Sherry L. Dupere	Ohio State University	T. E. O'Connor
Karen B. Ekelman	Ohio State University	C. Peraino
Michael L. Garriott	Purdue University	D. Grahn
Carol S. Giometti*	University of Illinois at the Medical Center	N. G. Anderson
Ross E. Jones	Michigan State University	C. A. Reilly, Jr.
Gary Kaufman+	University of Chicago	C. A. Reilly, Jr.
Peter A. Lagocki	University of Chicago	Y. E. Rahman
James M. Leonardo	Temple University	M. J. Peak
Shin-ichi Murao	Kobe University, Kobe, Japan	E. Huberman
Jennifer G. Peak	Rhodes University	H. E. Kubitschek
Paul R. Reynolds	University of Virginia	H. E. Kubitschek
Richard S. Rosenberg	University of Chicago	C. F. Ehret
Herbert M. Schwartz	Brandeis University	M. MacCoss
Reto A. Schwendener	Swiss Federal Institute of Technology, Zurich, Switzerland	Y. E. Rahman
Michael T. Short	University of Illinois at the Medical Center	M. Schiffer
Ido Simon	Weizmann Institute of Science, Rehovat, Israel and the University of Iowa	E. Huberman

*Now Assistant Biologist.

+Research Associate.

Fred J. Stevens*	Michigan State University, East Lansing	M. Schiffer
Fumio Suzuki	Kanazawa University	M. M. Elkind
Karen E. Willard	Virginia Polytechnic Institute and State University	N. G. Anderson

FACULTY RESEARCH PARTICIPATIONS

Argonne and the Division have long had a tradition of involving faculty members from colleges and universities, and staff from industrial laboratories, in Argonne activities. During 1981, there were in the Division two Visiting Scientists and fifteen appointments from the Faculty Research Participation Program and the Faculty Research Leave at Argonne Program. The two latter programs are administered through the Argonne Division of Educational Programs. The program for Faculty Research Leave at Argonne was initiated on a pilot scale in 1980; during 1981 three faculty members participated in the program in the Division. These appointments enable faculty members to participate in the research activities of the Laboratory in order to broaden their perspectives, and they allow university and Argonne Scientists to develop rapport and explore mutual interests. The names of the Visiting Scientists, Faculty Research Participants, and Faculty Research Leave at Argonne participants during 1981, their schools, and their staff sponsors were as follows:

Ehud Ben-Hur	Nuclear Research Center, Israel	M. M. Elkind
William E. Boernke+	Nebraska Wesleyan University	C. Peraino
Harold C. Furr	Illinois Benedictine College	M. H. Bhattacharyya
Donald K. Jasper	Illinois Institute of Technology	T. E. Fritz
Michael L. Johnson	University of Illinois at the Medical Center	M. MacCoss
Margaret M. Jonah	Rosary College	Y. E. Rahman
Alice Kenyon	Bethune-Cookman College	C. A. Reilly, Jr.
Ellen H. Lau	College of DuPage	Y. E. Rahman
DeSales Lawless	Fordham University	Y. E. Rahman
Eugene W. McArdle	Northeastern Illinois University	C. F. Ehret
J. Emory Morris$^{\alpha}$	State University College of New York, Brockport	C. Peraino
George M. Mukunnemkeril	North Central College	T. M. Seed
Dennis Nyberg$^{\alpha}$	University of Illinois at Chicago Circle	C. F. Ehret
Daniel G. Oldfield	DePaul University	T. M. Seed
S. Peter Spragg+	University of Birmingham, Birmingham, England	N. G. Anderson
Robert W. Tuveson$^{\alpha}$	University of Illinois at Urbana	M. J. Peak
Hiroshi Utsumi	Kyoto University, Japan	M. M. Elkind

*Assistant Biophysicist, February 1981.

+Visiting Scientist.

$^{\alpha}$Scientist in Residence (Faculty Research Leave at Argonne appointee).

SUMMER BIOLOGY RESEARCH INSTITUTE

Nine students from nine different universities were enrolled during the Summer of 1981 in this graduate level program offered by the Division of Biological and Medical Research in cooperation with the Argonne Division of Educational Programs. In addition, nine students from the Summer Undergraduate Research Participation Program also benefitted from the lectures and activities of the Research Institute.

Dr. Tatsuo Matsushita served as organizer and coordinator of the Institute program. The 10-week program featured a lecture series covering a variety of topics, including two-dimensional electrophoretic mapping of proteins; hemopathology and other late effects of protracted gamma irradiation in dogs; incidence and repair of neoplastic transformation in cultured mammalian cells after irradiation with gamma rays and neutrons; characteristics of cell growth in bacteria; mammalian toxicology of coal combustion effluents; short-term screening assays in mammalian genetic toxicology; molecular effects of solar ultraviolet radiation; phospholipid prodrugs of nucleoside analogues; and antigens and metals associated with chromatins of normal and neoplastic tissues.

The lectures were given by Drs. N. L. Anderson, T. E. Fritz, M. L. Garriott, C. K. Hill, F. R. Kirchner, H. E. Kubitschek, M. MacCoss, T. E. O'Connor, M. J. Peak, and T. M. Seed.

The lectures were supplemented by informal discussions and visits to laboratories as appropriate. Each student spent the remainder of his time working in a laboratory of a staff member.

The graduate students, their schools, and their staff sponsors were as follows:

Joey V. Barnett	Indiana State University	C. F. Ehret
David J. Crowe	Miami University, Oxford, OH	M. L. Garriott
Thomas Frommel	Texas A & M	T. M. Seed
Lynda Lanning	Auburn University	T. E. Fritz
Mary Ann Nocera	State University of New York at Fredonia	C. A. Reilly, Jr.
Karl Rogers	University of Pittsburg	Y. E. Rahman
Robert J. Ross	Northern Illinois University	M. MacCoss
Gerald H. Thomsen	University of Tampa	C. F. Ehret
Joseph H. Yost	Creighton University	H. E. Kubitschek

OTHER GRADUATE PROGRAMS

Four graduate students were Laboratory Graduate Participants working in the Division on research for their Ph.D. degrees in a program administered and suported by the Division of Educational Programs. The Laboratory Graduate Participants, their schools, and their staff sponsors were as follows:

Steven H. Gray	University of Illinois at the Medical Center	M. MacCoss
Bruce Hammer	Northwestern University	M. MacCoss

Mary E. Schackelford	University of Illinois at Chicago Circle	M. H. Bhattacharyya
Frederick J. Tremmel	University of Iowa	C. A. Reilly, Jr.

A related program, called Thesis Parts, is also supported by the Division of Educational Programs. It enables graduate students to perform pertinent parts of their research at Argonne. In 1981, one student held this appointment in the Division:

Mary A. Turner	University of Missouri	M. J. Peak

In addition, two graduate students carried out research in the Division as Resident Student Associates, supported directly by the Division:

Kevin R. Rupprecht	University of Notre Dame	C. A. Reilly, Jr.
Karen E. Willard	Virginia Polytechnic Institute and State University	N. G. Anderson

UNDERGRADUATE TRAINING

During 1981, a total of 35 college undergraduates received training in the Division of Biological and Medical Research through the Spring, Summer, and Fall Undergraduate Research Participation Programs, sponsored by the Division of Educational Programs. The students, their schools, and their staff supervisors are listed below:

Spring Program

Joey V. Barnett	Indiana State University	C. F. Ehret
Maria Cannella	York College, Jamaica, NY	F. R. Kirchner
Bernard Crespi	University of Chicago	F. R. Kirchner
Michael Douglass	Central Washington University	C. S. Borso
Robert Hickey	Allentown College of St. Francis de Sales	T. E. O'Connor
John Hurley	Humboldt State University	H. E. Kubitschek
Eric Lawson	Rust College	M. J. Peak
Brenda Mallard	Tougaloo College	N. G. Anderson
Michael McCroskey	Maryville College, Maryville, TN	M. MacCoss
Leslie Miller	Canisius College	Y. E. Rahman
Amy Rock	University of Michigan	M. E. Ginevan
Roger Scott	Cheyney State College	C. F. Ehret
Wendy Taddei	Widener College	T. E. O'Connor
Gerald Thomsen	University of Tampa	C. F. Ehret
Suzanne Ward	University of Southern Colorado	M. J. Peak
Joseph Yost	Creighton University	H. E. Kubitschek

Summer Program

Bruce I. Berkoff	Princeton University	C. S. Borso
Kathryn Bretscher	Texas Christian University	M. M. Elkind
Kevin Cancelliere	Allentown College of St. Francis DeSales	T. E. O'Connor
Valerie Doggett	North Carolina State University at Raleigh	T. M. Seed
Karen Jacaruso	Rochester Institute of Technology	T. E. Fritz
Thomas J. Kurtz	Edinboro State College	T. M. Seed
Brodis J. LaMarr	Morris Brown College	M. J. Peak
Cheryl Neal	Rosary College	Y. E. Rahman
Andrew Scheinman	University of Illinois, Urbana	C. S. Borso

Fall Program

Kathleen T. Blankenship	Chatham College	F. R. Kirchner
Renee M. Bressette	College of St. Benedict	C. A. Reilly, Jr.
Susan G. Cady	Rhode Island College	Y. E. Rahman
Kevin Cancelliere*	Allentown College of St. Francis DeSales	T. E. O'Connor
Perry P. Guaglianone	University of California, Berkeley	H. E. Kubitschek
Andrew S. Gulczynski	Northeastern Illinois College	C. F. Ehret
Ellen D. Jarrell	Barry College	T. E. O'Connor
Brodis J. LaMarr*	Morris Brown College	M. J. Peak
Carolyn A. Malik	Case Western Reserve University	Y. E. Rahman
Chris T. Mizumoto	Pacific Union College	C. S. Borso
Lisa A. Nerad	University of Illinois, Urbana	M. J. Peak
Margaret M. Orth	Carroll College, Helena, MT	H. E. Kubitschek

*Also participant in the Summer Undergraduate Research Participation program.

JOINT ARGONNE-UNIVERSITY APPOINTMENTS

During 1981, 15 staff members held a total of 17 faculty appointments at universities in the Chicago area. These appointments usually comprise limited teaching activities at the graduate level, generally of a specialized nature, which involve regular contact with students. They have led to cosponsorship of graduate students and to collaborative research efforts with faculty members, some of which are described in this report.

The affiliations with Chicago area universities were as follows:

University of Chicago

Mortimer M. Elkind
Eliezer Huberman
Timothy E. O'Connor
Warren K. Sinclair

Loyola University

Maryka H. Bhattacharyya
Thomas E. Fritz

Northern Illinois University

Maryka H. Bhattacharyya
Thomas E. Fritz
Douglas Grahn
Herbert E. Kubitschek
Carl Peraino
Yueh-Erh Rahman
Christopher A. Reilly, Jr.
Warren K. Sinclair
John F. Thomson
Robert B. Webb

University of Health Sciences/Chicago Medical School

Antun Han

University of Illinois at the Medical Center

Malcolm MacCoss

24. DIVISIONAL SEMINARS, JANUARY 1981 THROUGH JUNE 1982

Through September 1981, the Division of Biological and Medical Research Seminar Committee consisted of D. A. Haugen, Chairman, T. E. Fritz, M. E. Ginevan, F. R. Kirchner, C. Peraino, and M. Schiffer. For the rest of 1981 and the first half of 1982, the Committee consisted of A. Han, Chairman, M. MacCoss, M. J. Peak, and M. H. Bhattacharyya.

The General Seminar Program for 1981 consisted of a combination of in-house and visiting speakers, selected on the basis of recommendations from the staff. In addition, a number of informal seminars in specialized subjects were held during the year and during the summer period when the general seminars were not scheduled; some of these informal talks are indicated in the following listing.

VISITING SPEAKERS

Dr. Herbert Rosenkranz, Department of Microbiology, New York Medical College, Valhalla, NY
"Nitroarenes, an Unusual Group of Environmental Mutagens and Carcinogens"
January 15, 1981

Professor Francesco Bresciani, Universita di Napoli, Instituto di Patologia Generale, Napoli, Italy
"Molecular Mechanism of Estrogen Action"
January 29, 1981

Dr. James D. Yager, Department of Pathology, Dartmouth Medical School, Hanover, NH
"Promotion of Hepatocarcinogenesis by Oral Contraceptive Steroids"
March 5, 1981

Dr. Chris Foote, Chemistry Department, University of California, Los Angeles, CA
"Singlet Oxygen in Biological Systems"
April 9, 1981

Dr. John H. Miller, Radiological Sciences Department, Battelle Pacific Northwest Laboratories, Richland, WA
"Modeling Effects of Excision Repair in the Survival of Human Cells Exposed to Coal-Related Chemical Carcinogens"
April 23, 1981

Dr. I. D. Kuntz, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA
"Protein Structure and Protein Surfaces"
May 14, 1981

Dr. Dudley T. Goodhead, MRC Radiobiology Unit, Harwell, Didcot, England
"Physical Aspects of Radiation-Induced Lesions for Killing, Mutation and Chromosome Aberrations in Mammalian Cells"
May 28, 1981

Dr. Charles Land, Environmental Epidemiology Board, National Cancer Institute, Bethesda, MD
"The Epidemiology of Radiation Carcinogenesis: Methological Aspects"
June 4, 1981

Dr. Simon Folkard, Laboratory of Experimental Psychology, University of Sussex, Sussex, England
"Circadian Rhythms and Human Performance"
September 10, 1981

Dr. Kathleen A. Stitzel, Laboratory for Energy-Related Health Research, University of California, Davis, CA
"Hemopathological Effects of Chronic Low Dose Gamma Irradiation"
September 16, 1981

Dr. J. M. Holland, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN
"Chronic Dermal Toxicity of Synthetic Petroleum Liquids"
September 17, 1981

Dr. James E. Trosko, Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI
"Inhibition of Intercellular Communication in Tumor Promotion: Mechanisms, Applications, and Implications"
October 22, 1981

Dr. Warren K. Sinclair, Chairman, National Committee on Radiation Protection and Measurements, Bethesda, MD
"Estimation of Radiation Risk and the Dosimetry of the Atomic Bomb Survivors at Hiroshima and Nagasaki"
November 12, 1981

Dr. Jeanne M. Manson, Kettering Laboratory, Institute of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH
"Mechanism of Nitrofen-Induced Teratogenicity in the Rat"
November 19, 1981

Dr. Paul B. Fisher, Columbia University College of Physicians and Surgeons, New York, NY
"Multiple Interactions and Adenovirus Transformation: A Model System for Studying Tumor Promotion and Progression"
January 7, 1982

Dr. Jonathan Greer, Department of Biological Sciences, Sherman Fairchild Center for the Life Sciences, Columbia University, New York, NY
"Model Structures of Blood Proteins, Haptoglobin-Hemoglobin and Blood Clotting Factor Xa-Prothrombin"
January 13, 1982

Dr. Alan Epstein, Department of Medicine, Medical Oncology Section, Northwestern Medical School, Chicago, IL
"Classification Scheme for Nuclear Proteins Based on Their Immunofluorescence Staining Patterns using Monoclonal Antibodies"
January 21, 1982

Professor Donald J. Fluke, Zoology Department, Duke University, Durham, NC
"Mutagenesis and Damage Inducible Functions in E. coli"
February 4, 1982

Dr. Niza Frenkel, Department of Biology, Cummings Life Science Center, University of Chicago, Chicago, IL
"The Herpes Simplex Virus Amplicon - A New Eukaryotic Virus Cloning Vector"
February 11, 1982

Dr. Margaret L. Kripke, Frederick Cancer Research Center, Frederick, MD
"Immunological Aspects of Tumor Induction by Ultraviolet Light"
March 11, 1982

Dr. Robert C. Nowinski, Genetics Systems Corporation, Seattle, WA
"Diagnosis of Herpes Virus and Other Sexually Transmitted Diseases in Humans with Monoclonal Antibodies"
March 17, 1982

Professor M. Roberfroid, Catholic University of Louvain, Brussels, Belgium
"Chemical Promotion of Hepatocarcinogenesis, Rapid Production, Isolation, and Biochemical Characterization of Hyperplastic Nodules"
March 23, 1982

Dr. Robert A. Weinberg, Scientific Center for Cancer Research, MIT, Cambridge, MA
"Oncogenes of Human Tumor Cells"
April 22, 1982

Dr. Daniel W. Nebert, Developmental Pharmacology Branch, NIH, Bethesda, MD
"Cloning the Genes Encoding Enzymes Which Metabolize Drugs and Chemical Carcinogens"
May 5, 1982

Dr. Thomas P. Coohill, Physics Department, Western Kentucky University, Bowling Green, KY
"Ultraviolet Action Spectroscopy in Mammalian Cells and Viruses"
May 13, 1982

Dr. Heinz Kohler, Department of Molecular Immunology, Roswell Park, Memorial Institute, Buffalo, NY
"Regulation of Immune Responses Thr[illegible]h Idiotypes"
May 20, 1982

Dr. A. Leonard, Center for Nuclear Energy, Brussels, Belgium
"Cytogenetic Effects of Ionizing Radiations in Somatic Cells from Experimental Mammals and Extrapolation to Man"
May 21, 1982

Dr. Hans-Georg Schweiger, Max-Planck Institute for Cell Research, Heidelberg, West Germany
"Recent Developments in the Molecular Biology of Acetabularia"
June 3, 1982

IN HOUSE SPEAKERS

Dr. C. A. Reilly, Jr., Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Development of Alternate Energy Sources: The Role of Bio-Medical Research"
January 8, 1981

Dr. Fred J. Stevens, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Crystallographic and Biochemical Studies of Immunoglobulin Structure"
March 19, 1981

Dr. Paul T. Cunningham, Analytical Chemistry Laboratory, Chemical Engineering Division, Argonne National Laboratory, Argonne, IL
"The Analytical Chemistry Laboratory: A Statement of Our Purpose and Capabilities"
April 2, 1981

Dr. Herbert Schwartz, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Use of ^{17}O-NMR to Study Hydrogen Bonding in Nucleosides"
May 21, 1981

Dr. Maryka H. Bhattacharyya, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Gastrointestinal Absorption of Cadmium and Lead in Mice: Influence of Pregnancy and Lactation"
September 24, 1981

Dr. Malcolm MacCoss, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"The Synthesis, Structural Aspects, and Biological Activity of Covalent Nucleoside-Phospholipid Conjugates - A New Type of Prodrug"
October 29, 1981

Dr. Carl Peraino, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Studies of Multistage Hepatocarcinogenesis"
December 3, 1981

Dr. T. E. O'Connor, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"New Findings on the Biochemistry and Immunology of Chromatins"
December 10, 1981

Dr. M. J. Peak, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Biological and Molecular Effects of Long Wavelength Ultraviolet Radiations"
January 28, 1982

Dr. Antun Han, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Mutation and Neoplastic Transformation of Mammalian Cells by Ultraviolet Light"
February 25, 1982

Dr. N. Leigh Anderson, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"The Human Protein Index"
March 25, 1982

Dr. Frederick R. Kirchner, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Mammalian Toxicity Studies on Coal Gasification Effluents"
April 1, 1982

Professor Robert W. Tuveson, Faculty Research Leave at Argonne appointee, and Department of Genetics and Development, University of Illinois, Champaign-Urbana, IL
"Genetic Control of Near-UV Sensitivity in E. coli"
April 15, 1982

Dr. Thomas M. Seed, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Hematological Responses Under, Continuous Low Dose Irradiation: Mechanisms of Accommodation and Possible Relationships to Leukemia Induction"
June 17, 1982

25. OUTSIDE TALKS BY DIVISIONAL STAFF DURING 1981*

Anderson, N. G. "The Human Protein Index." American Association of Clinical Chemists, Argonne National Laboratory, Argonne, IL, March 17, 1981.

Anderson, N. G. "Technology in the Year 2000." National Committee for Laboratory Standards Conference, Cherry Hill, NJ, March 19, 1981.

Anderson, N. G. "The Human Protein Index: Relationship to Genetic Engineering." Battelle Conference on Genetic Engineering, Reston, VA, April 6-10, 1981.

Anderson, N. G. "High Resolution Protein Separations." Research and Development, Beckman Instruments, Fullerton, CA, April 23-24, 1981.

Anderson, N. G. "The Human Protein Index." Lecture, Vanderbilt University, Nashville, TN, April 30, 1981.

Anderson, N. G. "Molecular Anatomy Program." National Cancer Advisory Board, Washington, DC, May 18, 1981.

Anderson, N. G. "2-D Electrophoretic Analysis of Proteins." University of Toronto, Toronto, Canada, June 8, 1981.

Anderson, N. G. "Molecular Cell Mapping." Research and Development Division, Mallinckrodt Inc., Scottsdale, AZ, June 11, 1981.

Anderson, N. G. "Clinical Chemistry - Glimpses into the Future." Purchasing Practices in the Clinical Laboratory, A User/Manufacturer Interface, sponsored jointly by the Scientific Apparatus Makers Association, American Association for Clinical Chemistry, and College of American Pathologists, Kansas City, MO, July 23-24, 1981.

Anderson, N. G. "The Human Protein Index." Behring Symposium, Vienna, Austria, August 31-September 6, 1981.

Anderson, N. G. "High Resolution Two-Dimensional Electrophoretic Studies of Human Lymphocytes." Life Science '81, New York, NY, November 3-4, 1981.

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*Not included in this listing are lectures given as part of courses in area universities or in the educational programs at Argonne National Laboratory.

Anderson, N. G. "The Human Protein Index." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

Anderson, N. G. "The Human Protein Index." Seminar, Department of Biology, The University of Chicago, Chicago, IL, December 7, 1981.

Anderson, N. G. "The Human Protein Index: A Parts List for Man." S. Natelson Award, Chicago Section, American Association of Clinical Chemistry, Chicago, IL, December 11, 1981.

Anderson, N. L. "A Survey of Some Genetic Control Mechanisms in the Human Lymphocyte Using 2-D Electrophoresis." Electrophoresis '81, Electrophoresis Society, Charleston, SC, April 7-10, 1981.

Anderson, N. L. "Human Leukocyte Protein Index." MRC Laboratory of Molecular Biology, Cambridge, England, June 10, 1981.

Anderson, N. L. "Human Leukocyte Protein Index." Dupont Central Research and Development, Wilmington, DE, July 13, 1981.

Anderson, N. L. "Two-Dimensional Electrophoresis." 1981 American Association for Clinical Chemistry Symposium, Kansas City, MO, July 22, 1981.

Anderson, N. L. "High Resolution Two Dimensional Electrophoretic Studies of Human Lymphocytes." Life Sciences '81, New York, NY, November 3-4, 1981.

Anderson, N. L. "Analysis of the Heat Shock Response in Human Cells Using 2-D Electrophoresis: An Example of the Integration of Different Types of Data through Use of a Protein Index." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

Anderson, N. L. "Human Leukocyte Protein Index." NIH Workshop on Markers of Human Cancer, Bethesda, MD, December 18, 1981.

Bhattacharyya, M. H. "Health and Environmental Effects of Near-Term Battery Cycles." 4th U. S. DOE Battery and Electrochemical Conference, Washington, DC, June 2-4, 1981.

Bhattacharyya, M. H. "Effect of Oxidation State, Feeding Regimen, and Species on Gastrointestinal Absorption of Plutonium." 26th Meeting of the Health Physics Society, Louisville, KY, June 21-26, 1981.

Bhattacharyya, M. H. "Bioavailability of Orally Administered Cadmium and Lead to the Mother, Fetus, and Neonate during Pregnancy and Lactation." 21st Hanford Life Sciences Symposium, Richland, WA, October 4-8, 1981.

Borso, C. S. "Use of a New Photodiode Array Detector for Small Angle Scattering Determinations of Biological Structures." FEBS Advanced Course No. 78, Current Methods in Structural Molecular Biology, NATO Advanced Study Institute, Maratea, Italy, May 3-16, 1981.

Cahill, A. L. "Chronobiological Consequences of Various Shift Work Schedules." XVth International Conference of the International Society for Chronobiology, Minneapolis, MN, September 13-18, 1981.

Carnes, B. A. "Multivariate Methods for Assessing Disease/Environment Association: Conceptual Versus Geographic Regions." 1981 Sims, EPA, SIAM Environmetrics Conference, Alexandria, VA, April 8-10, 1981.

Carnes, B. A. "Approaches to Problems of Collinearity and Dimensionality in Studies of Disease-Environment Associations." Louisiana Tech University, Ruston, LA, November 2, 1981.

Collins, J. J. "The Use of a Demographic Model for Health Risk Assessments." Environmetrics '81, Washington, DC, April 6-8, 1981.

Derstine, P. L. "Relationship of Sister Chromatid Exchanges to Pyrimidine Dimer and DNA Break Induction with 365-nm Light." ICN-UCLA Symposium on Mechanisms of Chemical Carcinogenesis, Keystone, CO, February 22-March 1, 1981.

Doyle, D. E. "A Computerized Direct Data Entry System for Histopathology." C. L. Davis Seminar, Chicago, IL, March 14, 1981.

Doyle, D. E. "A Direct Data Entry System for Toxicologic Pathology." Meeting of the Society of Toxicologic Pathologists, Great Gorge, NJ, March 27, 1981.

Doyle, D. E. "Computerized Data Acquisition and Retrieval System for a Toxicologic Study in Dogs." American Association for Laboratory Animal Science, 10th Annual Southern Wisconsin-Chicago AALAS Interbranch Meeting, Madison, WI, September 11, 1981.

Duffy, P. H. "Effects of Intermittent 60-Hz Voltage Field Exposure: Recovery from Habituation in Mice and Arousal Response in Rats." 3rd Annual Meeting of the Bioelectromagnetics Society, Washington, DC, August 10-12, 1981.

Edwards, J. J. "The Use of Two-Dimensional Electrophoresis in the Search for Cancer Markers: Theory and Applications." Biochemistry Department, Texas Medical Center, Houston, TX, April 5, 1981.

Edwards, J. J. "Use of Schlieren Patterns for Position Location in Isoelectric Focusing Gels." Electrophoresis '81, Electrophoresis Society, Charleston, SC, April 7-10, 1981.

Edwards, J. J. "High Resolution Two-Dimensional Electrophoretic Studies of Human Lymphocytes." Life Science '81, New York, NY, November 3-4, 1981.

Edwards, J. J. "Proteins of Human Urine: Identification and Map Positions of Some Major Urinary Proteins." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

Ehret, C. F. "The Circadian Chronotype and the Chronobiotic Action of Foods, Drugs, and Hormones." American Medical Association, Chicago, IL, January 14, 1981.

Ehret, C. F. "Chemical Bases and Control of Biological Rhythms: Human Implications." Regulatory Cell Biology Lecture Series, Department of Zoology, University of Texas, Austin, TX, February 25, 1981.

Ehret, C. F. "Circadian Connections to Neurophysiology, Longevity, and Cognitive Function." Research Conference on Consciousness, Physiology and Longevity, Biology Department, Maharishi International University, Fairfield, IA, April 14, 1981.

Ehret, C. F. "The Nature and Role of Circadian Biological Rhythms in Animal Behavior." Northern Illinois University Animal Behavior Class, Lincoln Park Zoo, Chicago, IL, May 16, 1981.

Ehret, C. F. "Engineering the Biological Clock to Cope with Jet Lag and Shift Work." Thornton Community College, South Holland, IL, May 31, 1981.

Ehret, C. F. "Automated Data Acquisition Methods in Circadian Chronobiology." 2nd Gordon Research Conference in Chronobiology, Andover, NH, June 16, 1981.

Ehret, C. F. "On the Chronobiotic Action of Catecholamines and of Catecholamine Inhibitors in the Mammal." Toward Chronopharmacology, Nagasaki, Japan, July 27-28, 1981.

Ehret, C. F. "Clinical Applications in Circadian Chronobiology." Preventive Care Conference, Hinsdale Sanitarium and Hospital, Hinsdale, IL, September 18, 1981.

Ehret, C. F. "Circadian Connections to Jet Lag, to Shift Work, and to Orthochronal Medicine." Sigma Xi Lecture Series, Argonne National Laboratory, Argonne, IL, September 24, 1981.

Ehret, C. F. "Circadian Connections to Human Physiology: Chronohygiene for the Shiftworker." Meeting of the Human Factors Society, Toronto, Ontario, Canada, October 10, 1981.

Ehret, C. F. "Patterns of Shift Work in the Power Industry: The Need for Circadian Chronohygiene in Bioengineering at the Man-Machine Interface." CSNI Specialist Meeting on Operator Training and Qualifications, Charlotte, NC, October 12-15, 1981.

Ehret, C. F. "Circadian Clock Connections to Jet Lag, to Shift Work, and to Orthochronal Medicine." Sigma Xi Lecture, Borg-Warner Research Center Chapter, Des Plaines, IL, December 2, 1981.

Ekelman, K. B. "Regulation of Tyrosine Aminotransferase, Ornithine Aminotransferase and Serine Dehydratase by Serum and Amino Acids in Monolayer Cultures of Rat Liver Parenchyma Cells and in a Hepatoma-Derived Cell Line." Tissue Culture Association Meeting, 32nd Annual Meeting, Washington, DC, June 7-11, 1981.

Elkind, M. M. "Repair Processes in Radiation-Induced Neoplastic Transformation and Cell Killing." Conference on Normal Tissue Effects, Bethesda, MD, April 13-15, 1981.

Elkind, M. M. "Repair Processes in Radiation-Induced Neoplastic Transformation in Vitro." XVth International Congress of Radiology (ICR), Section II, Brussels, Belgium, June 24-July 1, 1981.

Elkind, M. M. "Linear Dose Dependencies and Repair Processes, Implications for Risk Estimates." Third International Conference on Environmental Mutagens, Tokyo and Mishima, Japan, September 21-26, 1981.

Fritz, T. E. "Hematological Responses of Beagles Exposed Continuously to Low Doses of ^{60}Co Gamma Irradiation." 10th Congress of the International Society for Experimental Hematology, Munich, Germany, August 23-27, 1981.

Gemmell, M. A. "A Comparison of Lymphocyte and Granulocyte Proteins Using Two-Dimensional Electrophoresis." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

Ginevan, M. E. "Human Birth Weight Patterns as an Indicator of Environmental Health: Comparison of Similar Counties in Upstate New York." Environmetrics '81, Washington, DC, April 6-8, 1981.

Ginevan, M. E. "Multivariate Methods for Assessing Disease/Environment Association." Environmetrics '81, Washington, DC, April 6-8, 1981.

Ginevan, M. E. "Human Birth Weight as an Indicator of Environmental Stress." Health Impacts of Different Sources of Energy, WHO, UNEP, IAEA Symposium, Nashville, TN, June 22-26, 1981.

Ginevan, M. E. "Approaches to Problems of Collinearity and Dimensionality in Studies of Disease-Environment Associations." 1981 DOE Statistical Symposium, Long Island, NY, November 4-6, 1981.

Giometti, C. S. "Analysis of Human Muscle Biopsies by Two-Dimensional Electrophoresis." Cold Spring Harbor Meeting on Molecular and Cellular Control of Muscle Development, Cold Spring Harbor, NY, September 8-13, 1981.

Giometti, C. S. "A Comparison of Cytoskeletal Proteins from Human Fibroblasts, Peripheral Blood Lymphocytes, and Transformed Lymphocytes (GM607) by Two-Dimensional Electrophoresis." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

Grahn, D. "Analysis of Reciprocal Chromosome Translocation Frequencies Induced in Spermatogonia of Mice Exposed to an Internally Deposited α-Emitter, External γ-Rays, and Fission Neutrons." 12th Annual Meeting of the Environmental Mutagen Society, San Diego, CA, March 7, 1981.

Grahn, D. "Biological Effects of Nuclear Radiation." IAEA 1981 Nuclear Power Course, Safety Analysis Review, Argonne National Laboratory, Argonne, IL, March 24, 1981.

Grahn, D. "Somatic Effects of Low Level Radiation Exposure." Marquette County Emergency Physicians and Marquette General Hospital, Marquette, MI, April 17, 1981.

Grahn, D. "Radiation Protection Concepts." IAEA Training Course in Planning, Preparedness, and Response to Radiobiological Emergencies, Argonne National Laboratory, Argonne, IL, October 1, 1981.

Grahn, D. "Biological Effects of Radiation." IAEA Training Course in Planning, Preparedness, and Response to Radiobiological Emergencies, Argonne National Laboratory, Argonne, IL, October 2, 1981.

Han, A. "The Role of Repair in Radiation-Induced Oncogenic Transformation of Mammalian Cells." The University Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, TX, January 29, 1981.

Han, A. Repair of Radiation Damage in Mammalian Cells." Loyola University, Chicago, IL, April 3, 1981.

Han, A. "Enhanced Neoplastic Transformation of Mouse C3H10T1/2 Cells by 12-O-Tetradecanoyl-phorbol-13-acetate following Exposure to X-Rays or to Emission Spectrum Neutrons." 29th Annual Radiation Research Society Meeting, Minneapolis, MN, May 31-June 4, 1981.

Han, A. "The Effect of Dose Rate and Tumor Promoter on Radiation-Induced Transformation of 10T1/2 Cells." Center for Human Oncology, University of Wisconsin, Madison, WI, November 16, 1981.

Han, A. "Mutation and Neoplastic Transformation of Mammalian Cells by Far and Near-UV Light." Donner Laboratory, Lawrence Berkeley Laboratory, Berkeley, CA, December 14, 1981.

Han, A. "Influence of Repair Processes and Promotion on Neoplastic Transformation of Mammalian Cells." British Columbia Cancer Research Foundation, Vancouver, BC, December 16, 1981.

Haugen, D. A. "Isolation and Identification of Mutagenic Primary Aromatic Amines Present in Materials from Synthetic Fuels Technologies." 6th International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH, October 27-29, 1981.

Hill, C. K. "Neoplastic Transformation at Low Dose Rates of ^{60}Co γ-Rays and Fission Neutrons." Illinois Institute of Technology, Department of Biology, January 10, 1981.

Hill, C. K. Five seminars given to Residents at Rush-Presbyterian St. Lukes Medical Center, Chicago, Il, February through April 1981.

Hill, C. K. "Neoplastic Transformation by Fission Neutrons: Is Repair of Transformation Damage Dose Rate Independent?" 29th Annual Radiation Society Meeting, Minneapolis, MN, May 31-June 4, 1981.

Huberman, E. "Control of Mutagenesis and Cell Differentiation in Rodent and Human Cells by Environmental Chemicals." Symposium on Organ and Species Specificity in Chemical Carcinogenesis, Raleigh, NC, March 2-4, 1981.

Huberman, E. "Activation of Chemicals to Mutagens for Mammalian Cells by Intact Hepatocytes." Symposium on Metabolic Activation, San Diego, CA, March 7, 1981.

Huberman, E. "Mutagen-Induced Resistance to Mycophenolic Acid in Hamster Cells Can Be Associated with Increased Inosine 5'-Phosphate Dehydrogenase Activity." American Association of Cancer Research Society Annual Meeting, Washington, DC, April 27-30, 1981.

Huberman, E. "Control of Cell Differentiation and Mutagenesis by Agents which Initiate or Promote Tumor Formation." Radiation Research Society Annual Meeting, Minneapolis, MN, May 31-June 4, 1981.

Huberman, E. "Studies in the Induction of in Vitro Cell Transformation, Mutagenesis and Differentiation by Chemical Carcinogens." Course on Genetic Toxicology held at the University of Mexico, Mexico City, Mexico, August 24-28, 1981.

Huberman, E. "Control of Cell Differentiation and Mutagenesis by Chemicals which Initiate or Promote Tumor Formation." Department of Microbiology, University of Chicago, Chicago, IL, October 20, 1981.

Kirchner, F. R. "Mammalian Toxicity Studies on Coal Gasification Effluents." Department of Immunology, University of Wisconsin at Madison, Madison, WI, February 2, 1981.

Kubitschek, H. E. "Bilinear Cell Growth." Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN, April 10, 1981.

Kubitschek, H. E. "Mutagenicity of Fly Ash in the Ames Salmonella Assay." Electric Power Research Institute Symposium on Biological Effects of Fly Ash, Palo Alto, CA, October 21, 1981.

Kubitschek, H. E. "Bilinear Cell Growth of Escherichia coli." Department of Microbiology, Indiana University Medical Center, Indianapolis, IN, November 6, 1981.

MacCoss, M. "Synthesis and Biological Activity of Novel Nucleoside-Phospholipid Prodrugs." 4th International Round Table on Nucleosides, Nucleotides and their Biological Applications, Antwerp, Belgium, February 4-6, 1981.

MacCoss, M. "^{17}O NMR as a Probe of Nucleoside H-Bonding." Experimental NMR Spectroscopy Conference, Asilomar, CA, April 5-9, 1981.

Matsushita, T. "Cytogenetics and Biochemical Studies of Sister Chromatid Exchange in Mouse Myeloma Cells." Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Co., March 3, 1981.

Matsushita, T. "Mouse Myeloma Sister Chromatid Exchange as a Short-Term Test for Carcinogens." Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, Co, March 4, 1981.

Matsushita, T. "Relative Sensitivities of Mammalian Sister Chromatid Exchange and Bacterial Mutation Frequency." Environmental Mutagen Society 12th Annual Meeting, San Diego, CA, March 5-9, 1981.

Matsushita, T. "Mouse Myeloma Sister Chromatid Exchange as a Short-Term Test for Carcinogens." Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Inc., Albuquerque, NM, March 11, 1981.

Nance, S. L. "Human Cellular Heat-Shock Proteins." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

O'Connor, T. E. "Activation of Chromatin Antigens of Neoplastic and Normal Rat Liver Tissues by Removal of Metallic Cations through Chelation." 21st Hanford Life Sciences Symposium, Richland, WA, October 4-8, 1981.

Peak, M. J. "Analysis of Weak Mutagenicity in the Ames Salmonella Mutagenicity Assay." 12th Annual Meeting of the Environmental Mutagen Society, San Diego, CA, March 5-9, 1981.

Peak, M. J. "Instrumentation for High Intensity Monochromatic UV-B and UV-A." NB5 UV-B Irradiation Workshop, Gaithersburg, MD, April 6-8, 1981.

Peak, M. J. "Protection of E. coli and Transforming DNA by Glycerol against Near-Ultraviolet Light Inactivation: Concentration Dependence, Action Spectra and Possible Mechanisms." 9th Annual Scientific Meeting of the American Society for Photobiology, Williamsburg, VA, June 14-19, 1981.

Peak, M. J. "Biological and Molecular Effects of Long Wavelength Ultraviolet Radiation." McDowell Cancer Network, University of Kentucky, Lexington, KY, December 1, 1981.

Peraino, C. "Studies of Initiation and Promotion in Liver Carcinogenesis." Department of Biological Sciences, DePaul University, Chicago, IL, May 22, 1981.

Peraino, C. "Histochemical Detection of Pre-neoplastic Hepatocyte Foci." NIEHS, National Toxicology Program, Research Triangle Park, NC, September 18, 1981.

Peraino, C. "Multistage Carcinogenesis." Department of Biology, University of Alabama, Tuscaloosa, AL, October 28, 1981.

Peraino, C. "Frontiers in Modern Biology." Department of Biology, University of Alabama, Tuscaloosa, AL, October 29, 1981.

Powers, M. R. "Human Milk Proteins." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

Rahman, Y. E. "Are Hepatocytes Phagocytic?" National Science Foundation, Washington, DC, January 26, 1981.

Rahman, Y. E. "Liposomes as Carriers for Drug Delivery." Graduate School of Arts and Sciences, Northern Illinois University, DeKalb, IL, April 10, 1981.

Rahman, Y. E. "Liposomes as Carriers for Water Soluble and Insoluble Metal Chelators." NATO Advanced Studies Institute, Cape Sounion Beach, Greece, June 24-July 5, 1981.

Rahman, Y. E. "Liposomes as Carriers for Water Soluble and Insoluble Metal Chelators: Application to Iron Overload in Thalassemia." 4th International Conference on Surface and Colloid Science, Jerusalem, Israel, July 5-10, 1981.

Rahman, Y. E. "Liposomes in Chelation Therapy." Conference on Frontiers in Liposome Research, San Francisco, CA, November 5-7, 1981.

Rahman, Y. E. "Differences in the Characteristics of Particle Uptake by Liver Parenchymal and Kupffer Cells." 21st Annual Meeting of the American Society for Cell Biology, Anaheim, CA, November 9-13, 1981.

Rahman, Y. E. "Targeted Drug Delivery--Tomorrow's Therapy." Physics Division Colloquium, Argonne National Laboratory, Argonne, IL, December 4, 1981.

Reilly, C. A., Jr. "Hydrocarbon Carcinogenicity." Health and Environmental Risk Analysis Program Annual Meeting, Alexandria, VA, February 6, 1981.

Reilly, C. A., Jr. "Health and Environmental Studies of Coal Gasification Process Streams and Effluents." EPA Symposium on the Environmental Aspects of Fuel Conversion Technology - VI, Denver, CO, October 27, 1981.

Rosenberg, R. S. "Arousals Caused by High Voltage AC Field Exposures." 1981 APSS Meeting, Hyannis, MA, June 17-21, 1981.

Russell, J. J. "Comparative Analysis of the Testicular Distribution and Retention and of the Genetic Effects of ^{239}Pu Alpha Radiation in $B6CF_1$/ANL and $(101xC3H)F_1$/ORNL Mice." 26th Meeting of the Health Physics Society, Louisville, KY, June 21-26, 1981.

Sacher, G. A. "Evolutionary Theory in Gerontology." AAAS Annual Meeting, Toronto, Ontario, Canada, January 4, 1981.

Schwartz, H. M. "^{17}O NMR as a Probe of Nucleoside H-Bonding." 22nd Experimental NMR Conference, Asilomar, CA, April 7-9, 1981.

Seed, T. M. "Sequential Change in Bone Marrow Architecture during Continuous Low Dose Gamma Irradiation." Scanning Electron Microscopy/1981 Symposia, Dallas, TX, April 16, 1981.

Seed, T. M. "Clonal Growth Patterns in Soft Agar Cultures of Radiation-Induced Canine Leukemia Cells." 10th Congress of the International Society for Experimental Hematology, Munich, Germany, August 23-27, 1981.

Short, M. "Preliminary X-Ray Crystallographic Studies of Bence-Jones Protein Loc." Mid-West Autumn Immunology Conference, Minneapolis, MN, October 18-23, 1981.

Taylor, J. "A Computerized System for Matching and Stretching Two-Dimensional Gel Patterns Represented by Parameter Lists." Electrophoresis '81, Electrophoresis Society, Charleston, SC, April 7-10, 1981.

Taylor, J. "Design of the TYCHO I Gel Analysis System and Initial Experience with the Human Leukocyte Protein Database." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Rochester, MN, November 15-18, 1981.

Tollaksen, S. L. "A New Approach to Biochemistry: Two-Dimensional Electrophoresis and Disease Diagnosis." American Chemical Society, Lake Forest, IL, February 6, 1981.

Tollaksen, S. L. "Use of Carbomylated Charge Standards for Testing Batches of Ampholyte Used in Two-Dimensional Electrophoresis." Electrophoresis '81, Electrophoresis Society, Charleston, SC, April 7-10, 1981.

Tolle, D. V. "Leukemia Induction in Beagles Exposed Continuously to ^{60}Co Gamma Irradiation: Hematopathology." 10th Congress of the International Society for Experimental Hematology, Munich, Germany, August 23-27, 1981.

Willard, K. E. "Two-Dimensional Gel Analysis of Human Leukocyte Proteins." Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, July 1, 1981.

Willard, K. E. "Clinical Applications of Two-Dimensional Gel Electrophoresis." Blood Bank and Department of Immunohematology, Ulleval Hospital, Oslo, Norway, August 27, 1981.

Willard, K. E. "Two-Dimensional Gel Analysis of Leukocyte Proteins from Patients with Rheumatoid Arthritis." Institute of Clinical Biochemistry, Rikshospitalet, Oslo, Norway, September 15, 1981.

Willard, K. E. "Analysis of Human Lymphocyte Proteins. III. A Marker for the Early Detection and Diagnosis of Human Infectious Mononucleosis." Clinical Applications and Developments in Two-Dimensional Electrophoresis, Mayo Clinic, Rochester, MN, November 15-18, 1981.

26. PUBLICATIONS APPEARING IN CALENDAR YEAR 1981

JOURNAL ARTICLES

Anderson, N. L. High Resolution Two-Dimensional Electrophoretic Mapping of Immunoglobulin Light Chains. Immunology Lett. 2, 195-199 (1981).

Binn, L. N., R. H. Marchwicki, E. H. Eckermann, and T. E. Fritz. Viral Antibody Studies of Laboratory Dogs with Diarrheal Disease. Am. J. Vet. Res. 42, 1665-1667 (1981).

Boernke, W. E., F. J. Stevens, and C. Peraino. Effects of Self-Association of Ornithine Aminotransferase on Its Physiochemical Characteristics. Biochemistry 20, 115-121 (1981).

Borso, C. S., and S. S. Danyluk. Application of a Directly Exposed Self-Scanning Photodiode Array as a Linear Position Sensitive Detector in a Small-Angle X-Ray Scattering Instrument. Rev. Sci. Instrum. 51, 1669-1675 (1980).

Bradley, M. O., B. Bhuyan, M. C. Francis, R. Langenbach, A. Peterson, and E. Huberman. Mutagenesis by Chemical Agents in V79 Chinese Hamster Cells: A Review and Analysis of the Literature (ORNL work). Mutat. Res. 87, 81-142 (1981).

Cahill, A. L., and C. F. Ehret. Circadian Variations in the Activity of Tyrosine Hydroxylase, Tyrosine Aminotransferase, and Tryptophan Hydroxylase: Relationship to Catecholamine Metabolism. J. Neurochemistry 37, 1109-1115 (1981).

Cahill, A. L., S. M. Ferguson, and C. F. Ehret. Chronotypic Induction of Tyrosine Aminotransferase by α-Methyl-p-Tyrosine. Life Sciences 28, 1665-1671 (1981).

Curtiss, J. R. B., and D. Grahn. Population Characteristics and Environmental Factors That Influence Level and Cause of Mortality/A Review. J. Environ. Pathol. Toxicol. 4, 471-511 (1980).

DeMark, B. R., and P. D. Klein. Ammonia Gas: An Improved Reagent for Chemical Ionization Mass Spectrometry of Bile Acid Methyl Ester Acetates. J. Lipid Res. 22, 166-171 (1981).

Dixon-Davis, D. K., J. J. Collins, and D. Grahn. Mortality in 191 Counties near Energy Facilities: Use of Economic and Social Variables for Standardization. Environ. Res. 25, 106-125 (1981).

Edwards, J. J., and N. G. Anderson. The Nature of Observed Schlieren Patterns in Isoelectric Focusing Gels and Their Use for Position Location of Banded Proteins. Electrophoresis 1981 2, 161-168 (1981).

Edwards, J. J., S. L. Tollaksen, and N. G. Anderson. Proteins of Human Semen. I. Two-Dimensional Mapping of Human Seminal Fluid. Clin. Chem. 27, 1335-1340 (1981).

Ekelman, K. B., and C. Peraino. Effects of Feeding and Lighting Stimuli on the Synthesis of Ornithine Aminotransferase and Serine Dehydratase in Rat Liver. Arch. Biochem. Biophys. 209, 677-681 (1981).

Ezra, F. S., M. MacCoss, and S. S. Danyluk. Influence of Uracil Photohydrate Formation on the Conformational Properties of Heterodinucleoside Monophosphates. Biopolymers 19, 1983-2002 (1980).

Finkel, M. P. The Argonne Monohousing System: A Better Mouse Cage. Lab. Animal 10, 58-64 (1981).

Gerson, S., R. J. M. Fry, W. E. Kisieleski, and A. R. Sallese. Cell Renewal in Noncornified and Cornified Buccal Epithelium in the Rabbit. J. Invest. Dermatol. 74, 192-196 (1980).

Ginevan, M. E. A Poisson Trials Approach to Interpopulation Comparisons of Cause of Death Data. Environ. Res. 25, 147-159 (1981).

Ginevan, M. E. Reply to R. Bertell (Letter to the Editors). Health Phys. 41, 422-424 (1981).

Griego, V. M., G. C. Matsushita, and T. Matsushita. Near-UV Induction of Sister Chromatid Exchanges under Aerobic and Anaerobic Conditions. Photochem. Photobiol. 34, 51-55 (1981).

Griego, V. M., R. B. Webb, and T. Matsushita. Dependence on Stage of Growth of Mammalian Cell Sensitivity to Near-UV Irradiation. Photochem. Photobiol. 33, 211-214 (1981).

Hagan, M. P., and M. M. Elkind. Recovery after Exposure to Near-Ultraviolet Light of Cells Containing 5-Bromodeoxyuridine. Biophys. J. 34, 367-374 (1981).

Hass, B. S., E. E. Brooks, K. E. Schumann, and S. S. Dornfeld. Synergistic, Additive, and Antagonistic Mutagenic Responses to Binary Mixtures of Benzo(a)pyrene and Benzo(e)pyrene as Detected by Strains TA98 and TA100 in the Salmonella/Microsome Assay. Environ. Mut. 3, 159-166 (1981).

Hass, B. S., and R. B. Webb. Photodynamic Effects of Dyes on Bacteria: IV. Lethal Effects of Acridine Orange and 460- or 500-nm Monochromatic Light in Strains of Escherichia coli That Differ in Repair Capability. Mutat. Res. 81, 277-285 (1981).

Haugen, D. A. Biphenyl Metabolism by Rat Liver Microsomes: Regioselective Effects of Inducers, Inhibitors, and Solvents. Drug Metab. Disp. 9, 212-218 (1981).

Haugen, D. A., M. J. Peak, and C. A. Reilly, Jr. Use of Nitrous Acid-Dependent Decrease in Mutagenicity as an Indication of the Presence of Mutagenic Primary Aromatic Amines: Non-Specific Reactions with Phenols and Benzo(a)pyrene. Mutat. Res. 82, 59-67 (1981).

Johnson, M. E. Apparent Hydrogen Bonding by Strongly Immobilized Spin-Labels. Biochemistry 20, 3319-3328 (1981).

Jones, C. A., P. J. Marlino, W. Lijinsky, and E. Huberman. The Relationship between the Carcinogenicity and Mutagenicity of Nitrosamines in a Hepatocyte-Mediated Mutagenicity Assay (ORNL work). Carcinogenesis 2, 1075-1077 (1981).

Kern, F., Jr., G. T. Everson, B. DeMark, C. McKinley, R. Showalter, W. Erfling, D. Z. Braverman, P. Szczepanik-van Leeuwen, and P. D. Klein. Biliary Lipids, Bile Acids, and Gallbladder Function in the Human Female: Effects of Pregnancy and the Ovulatory Cycle. J. Clin. Invest. 68, 1229-1242 (1981).

Kirchner, F. R., J. O. Hutchens, P. C. Brennan, and W. P. Norris. Redispersion of Fly Ash from a Liquid Nitrogen Suspension. J. Aerosol Sci. 12, 39-47 (1981).

Kubitschek, H. E. Bilinear Cell Growth of Escherichia coli. J. Bacteriol. 148, 730-733 (1981).

Kubitschek, H. E., and R. J. Doyle. Growth Delay Induced in Escherichia coli by Near-Ultraviolet Radiation: Relationship to Membrane Transport Functions. Photochem. Photobiol. 33, 695-702 (1981).

Lau, E. H., E. A. Cerny, and Y. E. Rahman. Liposome-encapsulated Desferrioxamine in Experimental Iron Overload. Br. J. Haematol. 47, 505-518 (1981).

MacCoss, M., C. F. Ainsworth, G. Leo, F. S. Ezra, and S. S. Danyluk. Conformational Characteristics of Rigid Cyclic Nucleotides. 3. The Solution Conformation of β-Lyxonucleoside Cyclic 2',5'- and 3',5'-Monophosphates and of α-Arabinonucleoside Cyclic 2',5'-Monophosphates. Implications for Evaluation of the Solution of Properties of Nucleoside Analogues. J. Am. Chem. Soc. 102, 7353-7361 (1980).

Matsushita, T., E. K. Ryu, C. I. Hong, and M. MacCoss. Phospholipid Derivatives of Nucleoside Analogs as Prodrugs with Enhanced Catabolic Stability. Cancer Res. 41, 2707-2713 (1981).

McArdle, E. W., B. L. Bergquist, and C. F. Ehret. Structural Changes in Tetrahymena rostrata during Induced Encystment. J. Protozool. 27, 388-397 (1980).

Millington, W. F., G. T. Chubb, and T. M. Seed. Cell Shape in the Alga Pediastrum (Hydrodictyaceae; Chlorophyta). Protoplasma 105, 169-176 (1981).

Peak, J. G., C. S. Foote, and M. J. Peak. Protection by DABCO against Inactivation of Transforming DNA by Near-Ultraviolet Light: Action Spectra and Implications for Involvement of Singlet Oxygen. Photochem. Photobiol. 34, 45-49 (1981).

Pearson, T., and L. Anderson. Analytical Techniques for Cell Fractions. XXVIII. Dissection of Complex Antigenic Mixtures Using Monoclonal Antibodies and Two-Dimensional Gel Electrophoresis. Anal. Biochem. 101, 377-386 (1980).

Peraino, C., C. F. Ehret, K. R. Groh, J. C. Meinert, and G. D'Arcy-Gomez. Phenobarbital Effects on Weight Gain and Circadian Cycling of Food Intake and Body Temperature. Proc. Soc. Exp. Biol. Med. 165, 473-479 (1980).

Peraino, C., E. F. Staffeldt, and V. A. Ludeman. Early Appearance of Histochemically Altered Hepatocyte Foci and Liver Tumors in Female Rats Treated with Carcinogens One Day after Birth. Carcinogenesis 2, 463-465 (1981).

Raedsch, R., A. F. Hofmann, and K.-Y. Tserng. Separation of Individual Sulfated Bile Acid Conjugates as Calcium Complexes Using Reversed-Phase Partition Thin-Layer Chromatography. J. Lipid Res. 20, 796-800 (1979).

Ryu, E. K., and M. MacCoss. New Procedure for the Chlorination of Pyrimidine and Purine Nucleosides. J. Org. Chem. 13, 2819-2823 (1981).

Schoeller, D. A., P. D. Klein, J. B. Watkins, T. Heim, and W. C. MacLean, Jr. ^{13}C Abundances of Nutrients and the Effect of Variations in ^{13}C Isotopic Abundances of Test Meals Formulated for $^{13}CO_2$-Breath Tests. Am. J. Clin. Nutr. 33, 2375-2385 (1980).

Sinclair, W. K. Hydroxyurea Revisited: A Decade of Clinical Effects Studies. Int. J. Radiat. Oncol. Biol. Phys. 7, 631-637 (1981).

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27. STAFF AND FUNDING AGENCIES*

LOW LEVEL RADIATION

Neutron and Gamma Ray Toxicity Studies

DOE: Carcinogenesis (HA-02-02-01) ANL-60300 "Neutron and Gamma-Ray Toxicity Studies"

NRC: "Relative Biological Effectiveness of Fission Neutrons and Gamma Rays at Occupational Levels" ANL-8M456

Regular Staff

J. F. Thomson (Senior Biologist)
Principal Investigator (to 6/82)
D. Grahn (Senior Biologist)
Principal Investigator (from 6/82)

K. A. Allen (Scientific Assoc.)
G. L. Holmblad (Scientific Assoc.)
J. L. Hulesch (Scientific Asst.)
L. S. Lombard (Vet. Pathologist)
V. A. Ludeman (Scientific Asst.)
A. R. Sallese (Scientific Asst.)
E. V. Staffeldt (Scientific Asst.)
J. E. Trier (Engineering Asst.)
B. J. Wright (Scientific Assoc.)

Radiation Toxicity in Dogs

DOE: Carcinogenesis (HA-02-02-01) ANL-63100 "Radiation Toxicity in Dogs"

NRC: Interagency Agreement, "Late Effects of Protracted Irradiation in Dogs" ANL-8D407

Regular Staff

T. E. Fritz (Vet. Pathologist)
Principal Investigator

D. E. Doyle (Scientific Asst.)
L. V. Kaspar (Scientific Assoc.)
W. G. Keenan (Scientific Assoc.)
L. S. Lombard (Vet. Pathologist)
C. M. Poole (Veterinarian)
A. R. Sallese (Scientific Asst.)
T. M. Seed (Biologist)
D. V. Tolle (Asst. Biologist)

*Abbreviations: DOE, Department of Energy; NCI, National Cancer Institute; NRC, Nuclear Regulatory Agency; NIAMDD, National Institutes of Arthritis, Metabolism and Digestive Diseases.

Staff listings and funding cover the period 1981 through April 1982. Participating staff from divisions other than the Division of Biological and Medical Research are not included.

Radiation-Induced Hemopathologies

DOE: Carcinogenesis (HA-02-02-01) ANL-63105 "Sensitive Indicators of Altered Target Cell Function In Chronic Toxicity Studies"

Regular Staff

T. M. Seed (Biologist)
Principal Investigator

L. V. Kaspar (Scientific Assoc.)

Radiation Effects in Cultured Mammalian Cells

DOE: Carcinogenesis (HA-02-02-01) ANL-61302 "Mutation and Transformation: New Cell Systems for Studies of Mechanisms"

Regular Staff

M. M. Elkind (Senior Biophysicist)
Principal Investigator (to 10/81)
A. Han (Biophysicist)
Principal Investigator (from 10/81)

DOE: Mutagenesis (HA-02-02-02) ANL-61300 "Mechanisms of Radiation-Induced Changes in Mammalian Cell Properties"

E. M. Buess (Scientific Asst.)
J. L. Dainko (Scientific Asst.)
P. J. Dale (Scientific Asst.)
C. M. Liu (Scientific Asst.)

NCI: "UV-X-Ray Interaction: Mutation and Transformation" ANL-85743

NCI: "Damage-Repair Studies Related to Mammography" ANL-85447

Temporary Staff

F. M. Buonaguro (Postdoc. Appointee)
C. K. Hill (Research Associate)
F. Suzuki (Postdoc. Appointee)

Mammalian Genetics

DOE: Mutagenesis (HA-02-02-02) ANL-62100 "Genetic Effects of High LET Radiations"

Regular Staff

D. Grahn (Senior Biologist)
Principal Investigator

DOE: "Relative Biological Effectiveness of Fission Neutrons and Gamma Rays at Occupational ANL-8M456

B. H. Farrington (Scientific Asst.)
J. J. Russell (Scientific Assoc.)

Temporary Staff

M. L. Garriott (Postdoc. Appointee)

CARCINOGENESIS

Mechanisms of Heaptocarcinogenesis

DOE: Carcinogenesis (HA-02-02-01) ANL-60400 "Carcinogenesis-Pollutant Interactions"

ANL-60402 "The Detection of Tumor Initiating, Promoting, and Synergistic Activity among Environmental Pollutants"

Regular Staff

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Mutagenesis and Cell Differentiation in Chemical Carcinogenesis

DOE: Carcinogenesis (HA-02-02-01) ANL-6041/ "Chemical Carcinogenesis Studies in Cultured Human and Rodent Cells"

Regular Staff

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NCI: Interagency Agreement, "Transformation of Syrian Hamster Embryo Cells by Carcinogenic Chemicals" ANL-8D408

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NCI: Interagency Agreement, "Malignant Cell Transformation and Mutagenesis Induced by Carcinogenic Chemicals" ANL-8D409

Temporary Staff

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Molecular and Genetic Mechanisms of Mutagenesis

DOE: General Life Sciences (HB-01) ANL-62201 "Molecular, Genetic, and Cellular Mechanisms of Environmental and Solar-UV Mutagens"

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Molecular and Cellular Effects of Solar Ultraviolet Light

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DOE: General Life Sciences (HB-01) ANL-62201 "Molecular, Genetic, and Cellular Mechanisms of Environmental and Solar-UV Mutagens"

Mechanisms of Damage and Repair by UVA, UVB, and UVC

Regular Staff

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DOE: General Life Sciences (HB-01) ANL-62201 "Molecular, Genetic, and Cellular Mechanisms of Environmental and Solar-UV Mutagens"

TOXICOLOGY

Metal Metabolism and Toxicity

Regular Staff

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E. S. Moretti (Scientific Asst.)
D. P. Peterson (Scientific Asst.)

DOE: Systems Damage (HA-02-02-03) ANL-61209 "Biological Effects of Metals in Developing Energy Technologies"

DOE: Health and Environmental Risk Analysis (HA-02-06) ANL-61210 "Assessment of the Health and Environmental Effects of Battery Energy Storage Systems"

NRC: "Reanalysis of Gastrointestinal Absorption Factors for Plutonium and Other Actinide Elements" ANL-8M447

Metal Toxicants: Carcinogenesis and Mitigation

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DOE: Carcinogenesis (HA-02-02-01) ANL-60414 "The Role of Metals as Cocarcinogens in the Induction of Malignant Tumors"

ANL-60400 "Carcinogenesis - Pollutant Interactions"

NIAMDD: "New Iron Chelator Delivery System for Cooley's Anemia" ANL-85830

Toxicology of Coal Gasification

Regular Staff

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DOE: Carcinogenesis (HA-02-02-01) ANL-60404 "Toxicological Evaluation of Coal Gasification Technology"

ANL-62200 "Genetic Toxicology of Coal Gasification"

DOE: Coal: Surface Coal Gasification (AA-85-25-05) ANL49533 "Process Development Unit Coal Gasification"

Toxicology of Coal Gasification: Chemical Characterization

Regular Staff

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DOE: Analytical Studies (HA-02-04-01) ANL-60407 "Fractionation/Characterization of Coal Gasification Process Streams for Biological Evaluation"

Neurobehavioral Chronobiology and Toxicology

Regular Staff

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DOE: Systems Damage (HA-02-02-03) ANL-61208 "Neurobehavioral Chronobiology and Toxicology"

DOE: General Life Sciences (HB-01) ANL-61200 "Detection and Characterization of Damage in Molecular, Cellular, and Physiological Systems"

DOE: Electric Energy Systems (AK-05-02) ANL-49185 "Biomedical Effects Associated with Energy Transmission Systems"

Biostatistics and Health Impacts of Energy Technologies

Regular Staff

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M. E. Ginevan (Asst. Statistician)
Principal Investigator

C. D. Brown (Scientific Assoc.)
B. A. Carnes (Asst. Biostatistician)
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N. Devine (Scientific Assoc.)

DOE: Human Health Studies (HA-02-01) ANL-68100 "Energy Production and Human Health"

DOE: Health and Environmental Risk Analysis (HA-02-06) ANL-68103 "Analysis of Health and Environmental Risks of Coal Gasification"

NRC: "Projection Models for Health Effects Assessment in Populations Exposed to Radioactive and Nonradioactive Pollutants" ANL-8M419

NRC: "Reanalysis of the Tri-State Leukemia Survey Data with Special Reference to the Leukemogenic Potential of Diagnostic X-Rays" ANL-8M420

THE HUMAN PROTEIN INDEX

The Human Protein Index

DOE: Systems Damage (HA-02-02-03)
ANL-61203 "Molecular Perturbations in Man Produced by Energy Related Pollutants"

DOE: Human Health Effects from Energy Generation (HA-02-01-01)
ANL-68112 "Development of New Technology for the Use of Isotopic Tracers in the Study of Human Health and Disease"

Regular Staff

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M. A. Gemmell (Scientific Asst.)
C. S. Giometti (Asst. Biologist)
S. L. Nance (Scientific Assoc.)
J. Taylor (Computer Scientist)
S. L. Tollaksen (Scientific Assoc.)

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K. E. Willard (Postdoc. Appointee)

BIOPHYSICS

Properties of Biological Molecules in Solution

DOE: General Life Sciences (HB-01)
ANL-61200 "Detection and Characterization of Damage in Molecular, Cellular, and Physiological Systems"

Regular Staff

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Structural Studies of Immunoglobulins

DOE: General Life Sciences (HB-01)
ANL-61200 "Detection and Characterization of Damage in Molecular, Cellular, and Physiological Systems"

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M. T. Short (Postdoc. Appointee)

Neutron and X-Ray Small Angle Scattering

Regular Staff

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Principal Investigator

DOE: General Life Sciences (HB-01)
ANL-61200 "Detection and Characterization of Damage in Molecular, Cellular, and Physiological Systems"