Division of Biological and Medical Research

Research Summary 1984-1985

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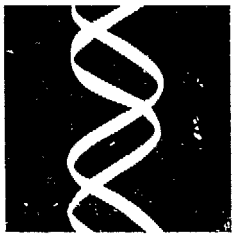

TABLE OF CONTENTS

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Division Director's Message
E. Huberman, Director vii

PART I. CARCINOGENESIS 1

1. Mechanisms of Hepatocarcinogenesis
C. Peraino, Principal Investigator 3

2. Role of Metals in Cocarcinogenesis and the Use of Liposomes for Metal Mobilization
Y. E. Rahman, Principal Investigator 7

3. The Control of Mutagenesis and Cell Differentiation in Cultured Human and Rodent Cells by Chemicals that Initiate or Promote Tumor Formation
E. Huberman, Principal Investigator 11

4. Radiation Effects in Mammalian Cells In Vitro
C. K. Hill, Principal Investigator 19

PART II. HUMAN RADIOBIOLOGY 25

5. Effects of Internally Deposited Alpha Emitters
J. Rundo, R. A. Schlenker, J. H. Stebbings, and R. E. Toohey, Principal Investigators 27

6. Health Effects of Industrial Exposure to Thorium
J. Rundo, J. H. Stebbings, and R. E. Toohey, Principal Investigators 35

7. Radon in Houses
R. E. Toohey, Principal Investigator 39

8. Alpha-Particle Effects on Cells
R. A. Schlenker, Principal Investigator 43

PART III. LOW-LEVEL RADIATION 47

9. Radiation Carcinogenesis and Radioprotectors
D. J. Grdina, Principal Investigator 49

10. Life Shortening, Tumor Induction, and Tissue Dose for Fission-Neutron and Gamma-Ray Irradiations
D. Grahn, Principal Investigator 53

11. Mammalian Genetics and Biostatistics
D. Grahn, Principal Investigator 59

12. Radiation Toxicity Studies in Dogs
T. E. Fritz, Principal Investigator 63

13. Hematopoiesis under Conditions of Chronic Toxicity
T. M. Seed, Principal Investigator 69

PART IV. MOLECULAR BIOLOGY 73

14. Molecular and Cellular Effects of Radiations
M. J. Peak, Principal Investigator 75

15. The Molecular Anatomy Program
N. G. Anderson and N. L. Anderson, Principal Investigators 81

16. Structural Studies of Proteins
M. Schiffer, Principal Investigator 89

17. Regulation of Cell Growth
H. E. Kubitschek, Principal Investigator 95

PART V. TOXICOLOGY 99

18. Toxicology of Energy-Related Complex Mixtures
C. A. Reilly, Jr., Principal Investigator 101

19. Chemical Characterization and Metabolism of Carcinogens
D. A. Haugen, Principal Investigator 105

20. Metal Metabolism and Toxicity
M. H. Bhattacharyya, Principal Investigator 109

21. Neurobehavioral Chronobiology: Actions of High-Strength 60-Hz Electric Fields on Circadian Rhythms
C. F. Ehret, Principal Investigator 115

PART VI. DIVISIONAL SUPPORT FACILITIES, SERVICES, AND ACTIVITIES 119

22. Support Facilities 121

23. Educational Activities 129

24. Divisional Seminars 135

25. Outside Talks by Divisional Staff 143

26. Professional Affiliations 159

27. Publications 163

28. Staff and Funding Agencies 175

AUTHOR INDEX 185

DIVISION DIRECTOR'S MESSAGE

Eliezer Huberman, Director

The Division of Biological and Medical Research (BIM) at Argonne National Laboratory conducts multidisciplinary research aimed at defining the biological and medical hazards to man from energy technologies and new energy options. These technically oriented studies have a strong base in fundamental research in a variety of scientific disciplines, including molecular and cellular biology, biophysics, genetics, radiobiology, pharmacology, biochemistry, chemistry, environmental toxicology, and epidemiology. Our staff is made up primarily of biologists, biochemists, and biophysicists with expertise in a variety of multidisciplinary areas, complemented by various support persons including pathologists, hematologists, toxicologists, veterinarians, statisticians, computer analysts, and engineering specialists.

On October 1, 1984, the Center for Human Radiobiology was transferred to BIM from the Environmental Research Division to become the Human Radiobiology Section, providing additional scientific expertise and specialized facilities to the Division. The Division is now organized into five scientific sections: Carcinogenesis, Human Radiobiology, Low-Level Radiation, Molecular Biology, and Toxicology. The sections are further divided into groups, as illustrated in the organizational chart on p. ix. The sectional organization emphasizes broad scientific relationships and encourages interactions and scientific collaboration among staff members with related interests. Each section holds regular seminars and discussion sessions, which persons from other sections are encouraged to attend.

This research summary is organized into six parts. The first five parts reflect the Divisional structure and contain the scientific program chapters, which summarize the activities of the individual groups during the calendar year 1984 and the first half of 1985. To provide better continuity and perspective, previous work is sometimes briefly described. Although the summaries are short, efforts have been made to indicate the range of research activities for each group. Each of the scientific chapters is followed by a list of relevant publications of the staff members for this same period. Also included are publications that were accepted for publication as of June 30, 1985.

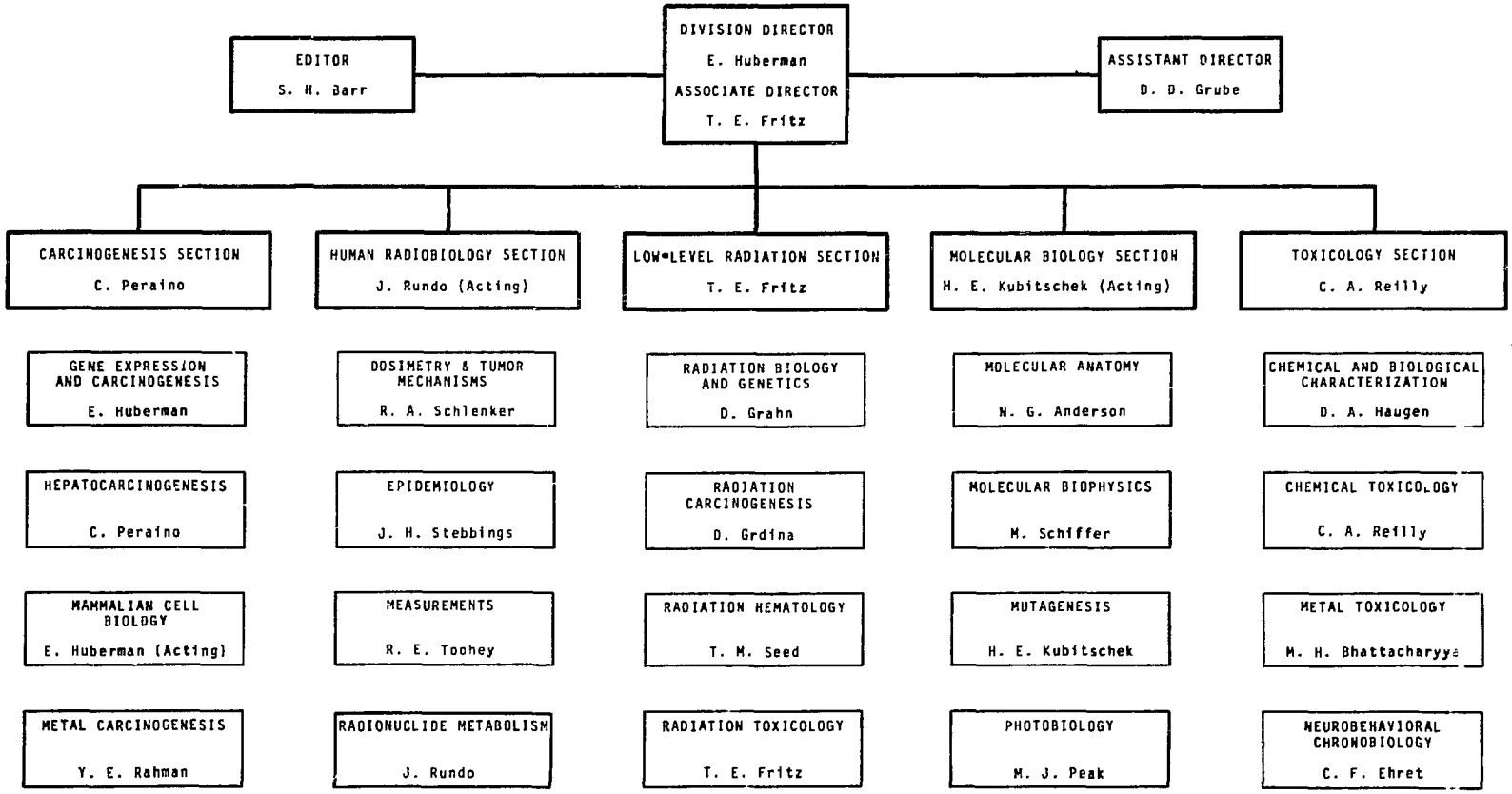
The final part of this report contains chapters that provide further information about the Division. They describe our specialized facilities, present the various graduate-

and undergraduate-level educational activities, list the Divisional seminars presented by visiting and in-house speakers and the principal outside talks given by staff members during 1984 and the first half of 1985, indicate the joint and adjunct appointments at colleges and universities and other professional activities of BIM staff members, and provide an inclusive listing of Divisional publications--journal articles, books or book chapters, and reports--from the beginning of January 1984 through June 1985. The last chapter lists the staff and the governmental agencies supporting each of the Divisional groups.

Divisional staff members have been active in the organization of international symposia and programs. Thomas M. Seed was one of the three organizers of the program, "The Ultrastructure of Radiation-Induced Injury in Cells and Tissues," which took place during the Scanning Electron Microscopy meeting in Philadelphia on April 20, 1984. "The Third International Symposium on Two-Dimensional Electrophoresis Protein Mapping," organized by Sandra L. Tollaksen, Norman G. Anderson, and N. Leigh Anderson, was held at Argonne from June 10 to 14, 1984. The Argonne National Laboratory International Symposium, "The Role of Chemicals and Radiation in the Etiology of Cancer," dedicated to the memory of Charles Heidelberger, was organized by a committee from the Division, consisting of Thomas E. Fritz, Colin K. Hill, Carol A. Jones, Carl Peraino, and myself. A volume incorporating papers from this symposium was published by Raven Press in July 1985.

During this period, BIM staff members were honored for a variety of accomplishments. William J. Eisler, Jr., received a 1985 University of Chicago (U. of C.) Outstanding Service Award. This award, established last year by the U. of C. Board of Governors for Argonne National Laboratory, recognizes individuals who have, through exceptional contributions in the areas of service or administration, furthered the goals and missions of Argonne. Christopher A. Reilly and Bruce A. Carnes were among the first recipients of the newly established Argonne National Laboratory Pacesetter Awards designed to recognize excellence in performance. The Pacesetter Award was presented to Chris Reilly for his outstanding and extraordinary effort in successfully developing a Laboratory-wide proposal for treaty verification. Bruce Carnes received his award for extraordinary effort expended in support of the National Cancer Institute/Department of Energy interagency agreement evaluating the effects of radiation on tumor induction and life shortening. Carol S. Giometti received the 1984 Young Clinical Chemist Research Award from the American Association for Clinical Chemistry for her work with tropomyosin variants. N. Leigh Anderson was selected by Science Digest as one of the nation's 100 most promising young scientists and was included in a comprehensive article in the December 1984 issue describing their research and views.

Future research efforts are planned to complement our existing programs, with continued strong emphasis on studies involving both the cellular and molecular mechanisms underlying the damage to DNA caused by chemicals and radiation and the processes involved in DNA repair. Research activities in molecular biology, biotechnology, and biophysics will be expanded, and interdisciplinary research collaboration, both within the Laboratory and with university and industrial participants, will receive continued strong encouragement. We look ahead to a productive and challenging year.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MEASUREMENTS
R. E. Toohey
RADIATION HEMATOLOGY
T. M. Seed
MUTAGENESIS
H. E. Kubitschek
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M. H. Bhattacharyya
METAL CARCINOGENESIS
Y. E. Rahman
RADIONUCLIDE METABOLISM
J. Rundo
RADIATION TOXICOLOGY
T. E. Fritz
PHOTOBIOLOGY
M. J. Peak
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Carcinogenesis

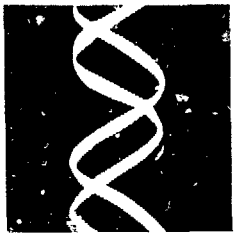
Gene Expression and Carcinogenesis
Hepatocarcinogenesis
Mammalian Cell Biology
Metal Carcinogenesis

The research conducted in the Carcinogenesis Section encompasses a wide variety of studies focused on the mechanisms underlying carcinogenesis induced by chemicals and radiation. The Hepatocarcinogenesis Group has developed a rat liver assay system; this system, in which biochemical markers identify small foci of preneoplastic cells, is used to test presumptive tumor initiators and promoters, analyze the sequential changes occurring in the liver during the neoplastic process, and investigate the nature of the gene-control processes that are functionally linked to the expression of neoplasia. (See Chapter 1.)

The role of toxic metals, specifically arsenic, in carcinogenesis is being investigated by the Metal Carcinogenesis Group in studies of the induction of skin cancer. This group is also examining new approaches to deliver chelating agents for the removal of toxic metals from their storage organs. Their research is described in Chapter 2.

To study organ specificity in chemical carcinogenesis, the Gene Expression and Carcinogenesis Group is testing carcinogens/initiators for their biotransformation into reactive metabolites by cultured cells derived from various organs. In addition, they are investigating the nature of carcinogen-induced genetic damage by using a family of defined genetic targets constructed by recombinant DNA techniques. Other studies involve the testing of tumor promoters for their ability to induce cell differentiation in various human cell lines. Chapter 3 presents this research. All of these studies are designed to identify critical cellular and biochemical events involved in the induction of neoplasia.

The mechanisms underlying radiation-induced cell death, mutation, and neoplastic transformation are currently being studied by the Mammalian Cell Biology Group in several cell culture systems. Of particular interest are the effects caused by low-dose-rate and fractionated irradiation. Inhibitors, promoters, and radioprotective compounds are used as a means of identifying the specific genetic or biochemical events involved in radiation-induced damage. (See Chapter 4.)



1. MECHANISMS OF HEPATOCARCINOGENESIS

C. Peraino, Principal Investigator
B. A. Carnes, J. M. Burcham, S. D. Vesselinovitch,*
J. A. Blomquist, V. A. Ludeman, A. M. Prapuolenis,
J. J. Russell, and E. F. Staffeldt

The objectives of our studies in hepatocarcinogenesis are to (1) analyze the mechanisms of liver tumor initiation and promotion and (2) develop our experimental liver tumorigenesis system as a rapid bioassay for carcinogens and promoters. In two complementary experimental approaches we are (1) determining the morphological and biochemical characteristics of hepatocytes undergoing the successive stages of tumorigenesis and (2) investigating the nature of the gene control processes that are functionally linked to the expression of neoplasia.

Our tumorigenesis studies involve administering a single dose of a tumor initiator to newborn rats, then, beginning at weaning, the continual feeding of tumor promoters. Histochemical analysis of the liver reveals the early formation of small foci of liver cells with biochemical markers like those present in most liver tumors. Histochemical information is then compared with tumor incidence data. This new treatment protocol has enhanced the ability of the liver system to detect environmental carcinogens and is facilitating the analysis of the critical changes in liver cells associated with the onset of neoplasia.

Our gene control studies involve comparing the control mechanisms for two adaptive hepatic enzymes that undergo dissimilar adaptive changes in tumors. Changes in enzyme synthesis rates, functional mRNA levels, and transcribable gene content associated with the responses of these enzymes to adaptive stimuli in normal liver are measured. Perturbations in these regulatory processes during the development of liver tumors can then be examined to gain insight into the essential molecular differences between tumor and normal tissue and to aid in designing short-term assays for identifying tumor-inducing chemicals.

In our carcinogenesis program, we continued to refine our experimental rat liver tumorigenesis system. Our principal observations were as follows:

1. The yield of altered hepatocyte foci was directly proportional to the

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amount of neonatally administered hepatocarcinogen (diethylnitrosamine) over an eightfold dose range (4-32 μg per gram body weight).

2. Full response to the hepatocarcinogen, in terms of focus production, was observed by 49 days after treatment, irrespective of dose, i.e., focus frequency reached dose-dependent plateaus at this interval and remained constant for the duration of the experiment (469 days). The maintenance of these plateau levels despite the continued ingestion of the promoter, phenobarbital, supports our prior conclusion that phenobarbital does not initiate neoplastic changes.

3. Although the yields of foci increased with increasing carcinogen dose, focus histochemical phenotypes, as indicated by the relative expressions of eight markers in the focus population, remained constant. This observation suggests that the factors determining the expression of histochemical markers in the foci are not sensitive to carcinogen dosage.

4. In carcinogen-treated rats, comparison of focus yields and phenotypes in the presence and absence of dietary phenobarbital showed that phenobarbital treatment increased focus production and, in contrast to carcinogen treatment, also increased the number of markers expressed per focus (phenotypic complexity). Thus, promotion apparently facilitates the emergence of the phenotypically more complex, i.e., less differentiated, foci that were initiated by prior carcinogen treatment but may require additional promoter-mediated events for expression.

5. Rat strain comparisons showed that Sprague-Dawley rats were more responsive than Fisher 344 rats with respect to focus and tumor production. Moreover, marker expression was somewhat higher and distributed differently in the Sprague-Dawleys than in the Fishers, suggesting that the strain differences may involve the mediation of expression rather than initiation.

6. Substitution of standard laboratory rat chow for our usual semisynthetic experimental diet produced a substantial reduction in focus yield, suggesting that the crude vegetable constituents of this diet may contain antineoplastic agents. This observation underlines the importance of using well-defined diets in tumorigenesis experiments.

7. Exposure of rats to gamma radiation induced small but significant numbers of foci; the incidence of these foci was significantly reduced if the rats were treated with the radioprotector S-2-(3-aminopropylamino) ethyl-phosphorothioic acid (WR-2721) 30 min before irradiation. Combining the radiation treatment with diethylnitrosamine administration synergistically increased focus yield, although this response declined with increasing doses of radiation. The magnitude of the synergism differed with different focus phenotypes. The results suggest that chemical carcinogens and gamma radiation affect different components of the mechanism of focus induction. (See also Chapter 9.)

8. Topical applications of coal-derived materials, beginning at weaning, to the backs of rats treated at birth with hepatocarcinogens substantially increased hepatic focus yields but had no effect in rats not treated with carcinogen. These observations

represent the first indication that systemic neoplasia may be influenced by dermal exposure to chemicals. (See also Chapter 18.)

9. Comparisons of the histochemical phenotypes of morphologically distinguishable carcinogen-induced hepatic lesions showed that the occurrences of the least-deviated morphologic changes were accompanied by substantial shifts in histochemical phenotypes toward greater complexity, in comparison with the phenotypes of histochemically detectable foci that were morphologically invisible. The various morphologic lesions, from the most to the least differentiated, had greater phenotypic resemblance to each other than to the purely histochemical foci, although the morphologic lesions did show successively increasing phenotypic complexity with increasing morphologic deviation from normalcy.

Collectively, our results have shown that a single carcinogen treatment followed by promotion produces a spectrum of lesions ranging from morphologically invisible but histochemically detectable to morphologically neoplastic. By studying the distribution of phenotypic properties in this population of lesions as a function of clearly defined modifying factors (e.g., gamma radiation, phenobarbital, rat strain) acting genetically or epigenetically, we can gain insight into (1) the relationship between different types and degrees of DNA damage and the initiation of specific types of cellular alteration, including neoplasia, and (2) the mechanisms by which the expression of these alterations is controlled.

Our studies of mechanisms controlling the synthesis of rat liver ornithine aminotransferase and serine dehydratase have progressed to the point that we can assay the functional mRNA levels for each enzyme with a cell-free translation system. Using such assays, we have observed that the synthesis of a given enzyme can be regulated at the level of mRNA transcription or at a post-transcriptional stage, depending on the nature of the adaptive stimulus. Refining this system should help us to determine the gene control sites susceptible to modulation under physiological states and during exposure to agents that influence neoplastic expression.

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1984

Peraino, C., E. F. Staffeldt, B. A. Carnes, V. A. Ludeman, J. A. Blomquist, and S. D. Vesselinovitch

Characterization of Histochemically Detectable Altered Hepatocyte Foci and Their Relationship to Hepatic Tumorigenesis in Rats Treated Once with Diethylnitrosamine or Benzo(a)pyrene within One Day after Birth

Cancer Res. 44, 3340-3347 (1984)

1985 through June

Peraino, C., E. F. Staffeldt, B. A. Carnes, V. A. Ludeman, J. A. Blomquist, and S. D. Vesselinovitch

Relationship of Histochemically Detectable Altered Hepatocyte Foci to Hepatic Tumorigenesis

Proceedings of a Symposium organized by the Hungarian Cancer Society and the IARC, Budapest, Hungary, May 16-18, 1983, Models, Mechanisms and Etiology of Tumour Promotion, M. Börzsönyi, N. E. Day, K. Lapis, and H. Yamasaki, eds., 1984, University Press, New York, NY, pp. 37-55

Accepted as of June 30, 1985

Ehret, C. F., C. Peraino, J. C. Meinert, and K. R. Groh

Circadian Manifestations of Barbiturate Habituation, Addiction, and Withdrawal in the Rat

Intl. J. Chronobiol.

Grdina, D. J., B. Nagy, C. K. Hill, R. L. Wells, and C. Peraino

The Radioprotector WR1065 Reduces Radiation-Induced Mutations at the HGPRT Locus in V79 Cells

Carcinogenesis

Peraino, C., and S. D. Vesselinovitch

Single-Hit Versus Multi-Hit Concept of Hepatocarcinogenesis

The Role of Chemicals and Radiation in the Etiology of Cancer, E. Huberman and S. H. Barr, eds., 1985, Raven Press, NY

2. ROLE OF METALS IN COCARCINOGENESIS AND THE USE OF LIPOSOMES FOR METAL MOBILIZATION

Y. E. Rahman, Principal Investigator
M. M. Jonah,* P. A. Lagocki,‡ and E. A. Cerny

The objectives of this program are to (1) develop and establish animal models for the investigation of metal tumorigenesis, (2) define the specific role of metal compounds in the process of tumor induction, and (3) develop new approaches for delivering chelating agents to effectively remove toxic metals from their storage organs.

Our current studies on the role of metals in tumor induction focus on arsenic. In studies of carcinogenesis of the skin, mice are first treated with a tumor initiator (physical or chemical) and then with a skin tumor promoter. Beginning at the time the mice are treated with initiator, they are also given arsenic at different levels in their drinking water. The mice are then monitored for the development of skin tumors. In hepatocarcinogenesis studies, rats are similarly exposed to arsenic in the drinking water, injected with a hepatocarcinogen, and then fed phenobarbital as a promoter. The livers are examined histochemically at intervals for the development of preneoplastic foci.

Two experiments testing the cocarcinogenic action of arsenic in the induction of skin cancer in SKH:hairless-1 mice have now been completed. Ultraviolet radiation (UV-B, 270-400 nm) was used as a physical skin tumor initiator; 7,12-dimethylbenz[a]anthracene (DMBA) was used as a chemical initiator. The following results were obtained:

1. Arsenic at nontoxic levels (< 40 mg/L) in the drinking water given concomitantly with ultraviolet radiation (UV) and 12-O-tetradecanoyl phorbol-13-acetate (TPA) (a known tumor promoter) treatment significantly increased the number of skin papillomas and squamous cell carcinomas.

2. Arsenic at subtoxic levels (49-60 mg/L) given concomitantly with UV followed by TPA treatment almost totally inhibited the induction of both papillomas and carcinomas.

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3. The number of papillomas increased very rapidly in mice given either DMBA + TPA or DMBA + TPA + arsenic (10 mg/L), while no papillomas were observed in groups of mice given one of the following: DMBA alone, arsenic alone, TPA alone, TPA + arsenic, and DMBA + arsenic.

4. Three weeks after the last TPA application, we observed an average of 10 papillomas per mouse in mice given DMBA + TPA, and 17 papillomas per mouse in the mice given DMBA + TPA + arsenic.

5. In the DMBA + TPA + arsenic group, 100% of the mice had skin papillomas by one week after the last TPA application.

6. About 5-7% of the papillomas assumed the morphology of squamous cell carcinomas in both groups of mice given either DMBA + TPA or DMBA + TPA + arsenic.

These results clearly show that arsenic plays an important role in skin carcinogenesis.

We have also initiated an experiment to define the specific role of arsenic in skin tumorigenesis. Arsenic could serve as a cocarcinogen, with its amplifying action on the initiator DMBA, or as an incomplete promoter (or second-stage promoter), with its action on the promoter TPA. To test the possibility that arsenic functions as an incomplete promoter, we used the classic two-stage protocol of promotion. Animals were first treated with DMBA, then low doses of TPA were applied for short periods. This treatment was followed by long-term treatment with various nontoxic levels of arsenic (2.5 to 20 mg/L) given in the drinking water. Preliminary results of this experiment indicate that arsenic given <u>after</u> initiation by DMBA does not enhance the induction of skin tumors, thus suggesting that arsenic does not function as a second-stage promoter. However, further experiments with larger numbers of mice are needed to confirm this finding.

In characterization studies of preneoplastic cells, we have compared the endocytic properties of normal and preneoplastic liver cells isolated from rats given diethylnitrosamine (DEN) followed by phenobarbital and injected with multiple doses of iron-dextran. Because normal hepatocytes selectively take up the iron complex, we were able to use a Percoll gradient to separate the preneoplastic cells from the denser normal hepatocytes. Using liposome uptake to test the endocytic capability of the cells, we showed the following:

1. Preneoplastic cells average ~20 μm in diameter, while normal hepatocytes average ~15 μm.

2. Both normal and preneoplastic hepatocytes clearly exhibit endocytic capabilities.

3. Given the same surface charge, large unilamellar liposomes are more effectively taken up by hepatocytes than are small unilamellar liposomes.

4. The endocytic capability of preneoplastic cells is significantly greater than that of normal cells.

5. Particle rigidity is an important factor for endocytic stimulation in preneoplastic hepatocytes.

6. Incubation at 4°C results in a more than 90% reduction in liposome uptake in both normal and preneoplastic cells.

To develop new ways to mobilize toxic metals from storage organs of animals, we are using two approaches to experimentally produce iron overloading in mice: (1) Iron overload in the reticuloendothelial system is produced by administration of heat-damaged ^{59}Fe-labeled red blood cells. (2) Iron overload in the liver parenchymal cells is produced by administration of purified ^{59}Fe-labeled ferritin. Lipid vesicles (liposomes) differing in size and lipid composition are used to encapsulate various metal chelators for delivery to these specific metal storage organs. Short-term cultures of liver parenchymal cells and peritoneal macrophages are used in in vitro studies of liposome-cell interactions.

Without exception, the chelating agents in clinical use today were introduced before 1960. Despite great research efforts by numerous investigators, no new chelating agents have been characterized or accepted into the clinic for more than 20 years. The ideal properties of chelating agents are well known, and ideal metal chelating agents have been synthesized; unfortunately, these synthesized agents are either ineffective or too toxic to be useful in vivo. Using the liposomes to deliver metal chelating agents, we have demonstrated that the ideal properties of a chelating agent can be expressed in the complex and competitive in vivo environment. We have not only demonstrated that the chelating effect of desferrioxamine (DF), the iron chelator of choice for clinical use, can be significantly enhanced by liposome encapsulation, we have also identified a new lipophilic chelator, N,N'-bis [2-hydroxybenzyl]-ethylenediamine-N,N'-diacetic acid (HBED), and demonstrated that it is far superior to DF.

In the past year, in collaboration with Ivan Schuller of the Material Science and Technology Division, we have also worked out an in vitro microanalysis method for iron in biological samples with the use of a superconducting quantum interference device (SQUID). An invention report has been filed with the Department of Energy on the basis of the preliminary results obtained from this study.

In summary, during this year we have further defined the role of arsenic in skin tumorigenesis. Arsenic does not function as a second-stage promoter; instead it appears to serve as a cocarcinogen. We have successfully isolated a population of preneoplastic focus cells from DEN-treated rats and have characterized some of the properties of this group of cells. We have also identified a new lipophilic metal chelator, HBED, and found it to be far superior to DF, the iron chelator of choice in clinical use since 1960. In addition, we have developed a new and rapid method for the microanalysis of iron in biological samples.

Publications

1984

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Liposomes Targeted to Cellular Receptors
Investigation of Membrane-Located Receptors, E. Reid, G. M. W. Cook, and D. J. Moore, eds., 1984, Plenum Publishing Corporation, NY, pp. 317-330

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The Effects of Charge and Size on the Interaction of Unilamellar Liposomes with Macrophages
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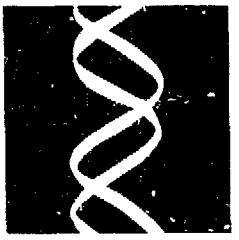

3. THE CONTROL OF MUTAGENESIS AND CELL DIFFERENTIATION IN CULTURED HUMAN AND RODENT CELLS BY CHEMICALS THAT INITIATE OR PROMOTE TUMOR FORMATION

E. Huberman, Principal Investigator
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F. Collart, J. P. Hardwick, Y. Homma, K. Kiguchi,
C. T. Oravec, F. Qian (Chien), M. F. Callaham,
C. B. Henning, and B. A. Sedita

The program objectives are to (1) study, in cultured human and rodent cells, the cellular and molecular events that govern the processes of tumor initiation and promotion in carcinogenesis induced by either radiation or chemicals and (2) establish simple and reliable cell systems for predicting the potential hazard of new chemicals introduced into our environment.

The studies involve two major types of experiments. In the first type, we use human and rodent cell culture assays to test carcinogens/tumor initiators for their ability to induce specific mutations and transform the cells to a malignant phenotype. For the various carcinogens, tumor initiators, and mutagens tested, results are related to their tissue specificity and their tumor potency in vivo. Mutations are characterized at defined DNA targets constructed by recombinant DNA techniques. In the second type of experiments, tumor promoters are tested by means of immunological, biochemical, and recombinant DNA techniques for their ability to initiate and alter gene expression in human cells that are either susceptible or resistant to the induction of cell differentiation by these agents. Differentiation is characterized by changes in morphological patterns and changes in functional proteins, enzymatic markers, and reactivity with monoclonal antibodies.

The mammalian cell-mediated mutagenesis assays that we have developed are useful for investigating some of the factors determining cell/organ specificity in chemical carcinogenesis. In these assays, mutable target cells (e.g., human P_3 teratocarcinoma cells), which cannot metabolically activate chemical carcinogens, are cocultured with primary cells derived from selected organs or tissues. Reactive metabolites generated by these primary cells are transferred to the susceptible target cells where their mutagenic activity can be expressed.

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Carcinoma of the colon accounts for approximately 10% of cancer deaths each year, whereas the duodenum and ileum are considerably less susceptible to tumor induction. To study the factors underlying this specificity, we have developed mutagenesis assays with rat colon, duodenum, or ileum cells and compared the activation in these assays of the colon-specific carcinogen, 1,2-dimethylhydrazine (DMH), with that in a rat-hepatocyte-mediated assay. Until now, colon cells were not believed to possess sufficient metabolizing enzymes to activate DMH; therefore, the critical metabolism was thought to occur in the liver with the active intermediates subsequently transported to the target colon cells via the blood. Our results indicate that colon cells, like hepatocytes do possess the necessary enzymes to metabolize DMH to mutagenic intermediates in the cell-mediated assay, whereas the duodenal and ileal cells do not. Thus, for DMH carcinogenesis, the particular susceptibility of the colon may be, in part, determined by the ability of the colon cells themselves to activate the carcinogen.

In addition to examining cell/organ specificity in chemical carcinogenesis, we are studying the nature of carcinogen-induced genetic damage. Our approach has been to develop a family of defined genetic targets that can be used to identify specific mutational changes induced by a carcinogen. The targets are derived from the gpt gene, which codes for an enzyme involved in the purine salvage pathway. This gene is being altered to contain defined mutations at a site that also eliminates susceptibility to a specific restriction nuclease. At this site, we introduce revertible mutations (frameshifts, base-pair transitions, base-pair transversions, or short duplications). These mutations will be incorporated into suitable vector DNAs and transfected into cells derived from the V79 and P_3 cell lines, which lack the mammalian analogue of the gpt gene product. A defined medium can then be used to select those cells in which the phenotype has been changed. Treatment with a carcinogen/mutagen may cause either an exact reversal of the initial mutation or produce a second mutation elsewhere that suppresses the enzyme defect. These two mutational pathways can be readily distinguished through the Southern blot technique. Thus, this family of genetically defined targets can be used to identify the type of genetic damage caused by a carcinogen/mutagen. We expect that this approach will be useful for studies on the mode of action of mutagens/carcinogens.

The biologically active metabolite of vitamin D_3, 1α,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$], regulates the biosynthesis of proteins involved in calcium and phosphate transport in the intestine and bone. This biological activity is mediated via the interaction of 1,25-$(OH)_2D_3$ with specific cellular receptors. Recently, we and others have reported that, in the human promyelocytic HL-60 leukemia cells, 1,25-$(OH)_2D_3$ can alter the pattern of cell differentiation in a manner resembling that of the tumor-promoting agents phorbol 12-myristate 13-acetate (PMA) and teleocidin, which also induce such differentiation in the HL-60 cells. We therefore decided to determine whether 1,25-$(OH)_2D_3$, like PMA, could enhance (promote) the transformation of cells that have been pretreated

(initiated) with known chemical carcinogens. For this study, we used the hamster embryo cell transformation assay in which the initiation-promotion sequence has been demonstrated. Our results indicate that this vitamin D_3 metabolite, in common with PMA, will not by itself effectively induce cell transformation. However, when administered subsequent to both direct-acting and proximate carcinogens/tumor initiators, both 1,25-$(OH)_2D_3$ and PMA will enhance the frequency of carcinogen-induced cell transformation. Furthermore, we observed that responsiveness of a particular embryo cell pool to the enhancing (promoting) effect of 1,25-$(OH)_2D_3$ was not necessarily correlated with a similar responsiveness to PMA. This difference may be partially because these two agents do not share the same receptors--a requirement for their biological activity.

An additional biological activity of tumor promoters is their ability to inhibit metabolic cooperation among cells. This property has led to the development of an in vitro assay with V79 cells to detect potential tumor promoters. In this assay, we have shown that 1,25-$(OH)_2D_3$, like PMA, is capable of inhibiting metabolic cooperation. Our studies also showed that in the V79 cells, as in human HL-60 leukemia cells, the binding site for 1,25-$(OH)_2D_3$ is different from that of the phorbol diesters.

Although 1,25-$(OH)_2D_3$ and PMA share several common biological properties, these chemicals differ in several other ways. Unlike PMA, 1,25-$(OH)_2D_3$ does not induce ornithine decarboxylase activity in quiescent BALB 3T3 cells nor does it promote papilloma formation in mouse skin. Furthermore, it appears to antagonize the promoting effect of PMA. Thus, although 1,25-$(OH)_2D_3$ possesses many of the characteristics associated with tumor promoters, it does not act like PMA in all cases and is presumably not a promoter of skin carcinogenesis. It may, however, act as a promoter of carcinogenesis in other target organs.

The analysis of the molecular basis for the action of tumor promoters can be aided by the availability of cells resistant to various tumor-promoter-induced cellular alterations, including the induction of cell differentiation. We have isolated variant cells from the HL-60 cell line that are resistant to induction of cell differentiation by PMA and related tumor promoters. We have also established that the acquired resistance of these cells to the phorbol diesters does not appear to result from an alteration in either the number of binding sites or the binding affinity of the phorbol diester to its receptors. Because receptor mobility, which is profoundly affected by membrane lipid fluidity, appears to play an important physiological role in a number of biological systems, we, together with colleagues from Columbia University, explored the dynamics of membrane lipids in the differentiation-susceptible and -resistant HL-60 cells using a series of membrane-permeant and membrane-impermeant fluorophores.

The probe, 1,6-diphenyl-1,3,5-hexatriene, can penetrate the plasma membrane and intercalate in the lipids of both leaflets (i.e., inner and outer) of the plasma membrane, as well as in organellar membranes. Using this probe, we found that the resistant

cells have a lower lipid fluidity than the susceptible HL-60 cells. In contrast, no difference in membrane lipid fluidity was observed when we compared the susceptible and resistant cells by using a series of membrane-impermeant fluorophores that incorporate only into the outer hemileaflet of the plasma membrane.

These observations suggest that the ability of HL-60 cells to respond to PMA may be affected by the physical state of the plasma membrane lipids and that the resistant phenotype is associated with decreased fluidity of either the inner leaflet of the plasma membrane and/or of the cytosolic organellar membranes.

Binding to high-affinity and saturable receptors initiates the biological activity of the phorbol diesters. This binding, in turn, activates a calcium- and phospholipid-dependent protein kinase, termed protein kinase C. Recent studies have demonstrated that both the phorbol diester receptor and protein kinase C reside in a single protein. Thus, it is possible that protein phosphorylation induced by these agents is the key element that initiates the differentiation processes in the HL-60 and perhaps other susceptible cells.

To identify such phosphorylated proteins, we collaborated with members of the Molecular Anatomy Group (see Chapter 15) to analyze, by two-dimensional gel electrophoresis systems, the patterns of the PMA-induced phosphorylated proteins in the HL-60 cells that are either susceptible or resistant to induction of cell differentiation by phorbol diesters. The HL-60 cells were incubated with PMA for only 30 min to allow the detection of protein phosphorylation associated with the induction rather than with the expression of cell differentiation.

Increased phosphorylation of at least 10 different proteins was observed in the differentiation-susceptible HL-60 cells following the brief incubation with PMA. The differentiation-resistant cells also exhibited increased levels of protein phosphorylation after PMA treatment, but the level of phosphate incorporation reached only the level of the untreated susceptible HL-60 cells and thus fell far short of the level observed in these cells after PMA treatment. The resistant cells may thus contain an ineffective protein kinase C, which is also the phorbol diester receptor. On the basis of our previous studies, we believe that the acquired resistance of the HL-60 cell variant to phorbol diesters does not appear to result from an alteration in either the number of binding sites or binding affinity of the phorbol diesters to their receptor (protein kinase C). Because these resistant cells, however, exhibit a reduced fluidity of the inner leaflet of the plasma membrane and/or of the cytosolic organellar membranes, we propose that the basis of the PMA resistance in these cells is a block in the signal transmission required for initiation of cell differentiation that occurs subsequent to receptor occupancy, which may involve the translocation of protein kinase C between different cellular compartments. Indeed, our initial results indicate that after PMA treatment the protein kinase C localizes in different cellular compartments in the differentiation-susceptible and -resistant HL-60 cells. These results may prove fundamental in understanding how tumor promoters exert their

pleiotropic biologic effects in diverse cell types.

In summary, our cellular and molecular studies provide further insight into the cellular and biochemical events involved in the control of cell differentiation and the development of neoplasia. Such information will lead to a greater understanding of the therapeutically exploitable differences between tumor and normal cells and can form the basis for the development of early biochemical indicators of neoplasia.

It is also anticipated that the work to develop and use the various assays with cultured human and rodent cells will lead to the development of a rapid and reliable method by which a broad spectrum of environmental contaminants can be tested for their tumor-initiating or -promoting activity, as well as their organ specificity.

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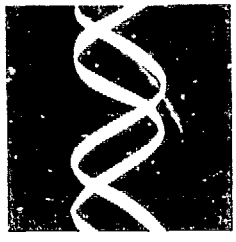

4. RADIATION EFFECTS IN MAMMALIAN CELLS IN VITRO

C. K. Hill, Principal Investigator
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E. M. Buess, and C.-M. Liu

The purpose of this research effort is to elucidate the mechanisms for the radiation-induced changes in mammalian cells that lead to cell death, mutation, neoplastic transformation, DNA damage, and chromosomal alterations. Of particular interest are the effects of low-dose-rate and fractionated irradiation on these end points with respect to the mechanisms whereby these effects are influenced by cellular repair processes, inhibitors, and promoters that act at the genetic or biochemical level. Radiations used include low linear-energy-transfer (LET) radiation such as ^{60}Co gamma rays and x-rays and high-LET radiation such as fission-spectrum neutrons from our Janus research reactor. (See Chapter 22 for a further description of the radiation facilities of the Division.)

The principal techniques that are used include (1) clonal growth of single cells to measure cell killing or induction of mutants, (2) transformation assays based on the identification of morphologically altered foci on an otherwise contact-inhibited layer of cells, (3) labeling of DNA-precursor macromolecules with radioisotopes and measuring the damage to DNA with sedimentation and elution techniques, and (4) karyotyping by modified Giemsa-staining techniques.

Neoplastic Transformation of Mammalian Cells.

During the period covered by this report, the Janus reactor was not available for research activities because of required engineering changes. However, we completed the analysis of results from previous experiments and initiated new experiments with ^{60}Co gamma rays.

Neutrons. In our previous studies, we suggested that the enhanced transformation observed after protracted exposures to low doses of neutrons might be due to misrepair and/or

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promotion of neutron damage. In experiments to examine these possibilities, we have shown the following:

1. Transformation frequency greatly increases when tetradecanoylphorbol-13-acetate (TPA), a known promoter, is added at 0.1 μg/mL to cultures 24 h after neutron irradiation.

2. After gamma-ray exposures, the increase in transformation frequency is about the same whether the TPA is given after high-dose-rate or low-dose-rate exposures. This finding suggests that a segment of the damage produced by gamma rays is independent of dose rate and that this damage can be promoted to form transformed foci. In cells that are not treated with promoter, this segment of damage is not expressed as transformed foci.

3. When neutron-irradiated cells are treated with TPA after irradiation, the increase in transformation frequency is dose-rate dependent. Proportionally less promotional effect of TPA occurs after irradiation with neutrons at a low dose rate. However, the absolute transformation frequency reached is about the same. We infer that part of the damage that would normally be expressed when TPA is added has already been expressed as a result of exposure to neutrons at a low dose rate.

4. Further, in studies using the antipromoter antipain, we have now clearly shown that when antipain is added after low-dose-rate neutron exposure, the transformation frequency returns to the level observed after high-dose-rate exposures. However, if we add antipain after high-dose-rate exposures, no effect on transformation frequency is observed. These results further support the hypothesis that neutrons delivered at a low dose rate act in a promoter-like fashion.

5. We are just beginning to examine the possibility that misrepair of some damage may facilitate the promoter-like effect.

<u>^{60}Co Gamma Rays</u>. Continuing our investigation of low-dose-rate effects on transformation, we have undertaken a major investigation with gamma rays given at 10 cGy/day (0.007 cGy/min) to cells grown at 37°C in an incubator. The protocol requires a 22-h irradiation day with a 2-h down time. The cells are grown in the radiation field in plastic T/75 flasks such that they maintain exponential-phase growth throughout the irradiation. The cells are passaged every five days, sampled for survival and transformation assays, and reseeded into fresh flasks. With this protocol, a total dose of up to 450 cGy can be accumulated during the useful life span of the cells.

The results so far are intriguing. Survival and transformation have been measured at total doses of 50, 150, 300, and 450 cGy, with or without addition of TPA (0.1 μg/mL) at the end of the radiation. Cells receiving radiation alone have a low level of transformation, a level, however, that is still significantly above background. This finding is consistent with earlier data that showed transformation to be reduced progressively as the dose rate of gamma radiation is reduced. However, when TPA is added, we find a significant increase in transformation--about 10- to 15-fold at all dose levels. This result suggests that potentially effective

damage can be propagated in the C3H 10T1/2 cells without being expressed. When a promoter is added, however, this damage will be expressed. Further experiments are in progress to determine if the sensitivity, survival, and/or growth characteristics of these cells change during such a prolonged irradiation.

In an ongoing collaboration with David Grdina of this Division, we have recently demonstrated that not only does the compound WR-1065 (N-[2-mercaptoethyl]-1,3-diaminopropane) protect against gamma-ray-induced cell killing and mutation when present during irradiation (see Chapter 9), but it also protects cells against transformation induced by gamma rays. Experiments are in progress to determine if WR-1065 will protect against transformation when it is administered following irradiation.

Mutation.

In parallel with the transformation studies, we are conducting an active study to investigate the effect of dose protraction on mutation as measured at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus by the induction of mutants resistant to 6-thioguanine. The data gathered with Chinese hamster cells, normal human fibroblasts (IMR91), and cells from a transformed human epithelial cell line (P_3) suggest that although the human cells are about twice as sensitive as the Chinese hamster cells to cell killing by ^{60}Co gamma rays and fission neutrons, the shapes of the curves for mutation induction are qualitatively similar. So far, with the dose rate reduced to either 0.5 cGy/min or 0.1 cGy/min for fission neutrons, there appears to be no significant change in mutation frequency. The estimated relative biological effectiveness (RBE) for induction of mutation by fission neutrons in the initial part of the induction curve is 15. Preliminary data for reduced dose rates of gamma rays show a clear reduction in mutation frequency for each cell line.

DNA Damage.

In the past year, we have begun new studies of DNA damage caused by radiation (single- and double-strand breaks and protein-to-DNA crosslinking) in mammalian cells using the sensitive DNA elution techniques. In collaboration with Meyrick Peak of this Division (see Chapter 14), we have performed calibration experiments using x-rays on V79 Chinese hamster cells and human P_3 cells; these experiments show that the alkaline elution techniques give results consistent with those obtained through sucrose sedimentation techniques, but with a resolution about 10 times better. For example, we can detect single-strand breaks after only 25 cGy of x-rays. We plan to use this technique to examine DNA damage in both our repair studies and our studies on the effects of radiation quality.

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Conference on Cell Transformation Assays, Research Triangle Park, NC, September 11-13, 1984

Human Radiobiology

Dosimetry and Tumor Mechanisms
Epidemiology
Measurements
Radionuclide Metabolism

The work conducted in the Human Radiobiology Section covers a number of diverse programs that relate to levels and effects of alpha-particle-emitting radionuclides in humans. The major project, which involves all four groups (Dosimetry and Tumor Mechanisms, Epidemiology, Measurements, and Radionuclide Metabolism), is the ongoing study of the late effects of radium in persons exposed either occupationally (especially as luminous dial painters) or medically to radium. This study is described in Chapter 5. A related epidemiological study, which makes use of the data base developed for the radium project, is being conducted to investigate the relationships among lung cancer, radon-daughter level, and cigarette smoking. Another related project, described in Chapter 6, is a study of the health effects of industrial exposure to thorium; this project makes use of many of the facilities and techniques developed for the radium study. The purpose of the radium project is to determine quantitative dose-response relationships and to use them to predict the toxicity of systemic deposits of such radioelements as plutonium and americium, which are produced in irradiated nuclear fuel. The thorium project focuses on determining toxicity because of the potential use of thorium in a nuclear fuel cycle and because inhaled thorium is regarded as an analogue of inhaled insoluble plutonium.

The radioactive gas radon is produced by the decay of radium; its rate of exhalation by persons containing the parent element can be a highly sensitive measure of skeletal radium content. In recent years, we have found that the radon level in the air of some houses can be so high that large radon reservoirs will build up in the bodies of persons living there due to dissolution in fats and fluids. These reservoirs are depleted by exhalation when residents leave home. Consequently, determination of exhaled radon can give highly misleading indications of the radium remaining in the body. As a result, we have undertaken a small project (described in Chapter 7) to determine the levels of radon in single-family housing. At the same time, a small project was initiated to investigate the relationship between an occupational atmosphere containing a high radon concentration and lung cancer in the radium dial painters. Finally, Chapter 8 presents research dealing with mutation, transformation, and survival of cells irradiated by low-energy alpha particles.

5. EFFECTS OF INTERNALLY DEPOSITED ALPHA EMITTERS

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The purposes of this ongoing multidisciplinary, multitask study are to (1) identify and quantify the human health effects of occupational exposures to radium, (2) use the health effects data from the radium study to predict responses to other alpha-emitting and/or bone-seeking radionuclides at occupational exposure levels and above, and (3) predict the effects of these radionuclides, specifically environmental radium and its daughters, at nonoccupational exposure levels. In man, internally deposited radium is known to cause bone sarcomas and carcinomas of nasal sinuses and mastoid air cells; suggestions have been made that it may also cause leukemias, multiple myeloma, and breast cancer. Acute and nonstochastic effects, including radiation osteitis and possibly cataracts, occur. Reproductive function may also be impaired by radium. The study of radium effects is important because the cohorts of former radium dial workers form the largest nonintrogenic human population exposed to long-lived alpha emitters (i.e., to radiation with high linear energy transfer) at unequivocally carcinogenic levels. Protection standards for plutonium, other alpha emitters, and other bone-seeking radionuclides have always been based in large part on the experience of these radium dial workers. Consequently, accurate determinations of radiation exposure and of tissue/cell dosimetry are essential. Other significant components of this study include the theoretical analyses of carcinogenic processes in humans, the determination of the effects of exposures to radon and radon daughters in a female population, and the setting of standards for radium in drinking water.

"Classical Tumors": Risk Assessment, Dose-Response and Data Collection.

The classical tumors unequivocally associated with internal exposure to ^{226}Ra and/or ^{228}Ra are bone sarcomas and carcinomas of the paranasal sinuses and mastoid air cells. These tumors have appeared in dial workers first exposed before 1926 and to those exposed medically before 1937, so the high-risk survivors are more than 70

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years old. There are ~50 subjects still alive with residual body burdens within the range that has yielded tumors in the past; additional tumors are therefore expected. Because of the short half-life (5.75 years) of ^{228}Ra, contemporary levels are very low in persons exposed to it 60 or more years ago. Consequently, it is difficult to determine these contemporary levels even by chemical analysis of bone obtained at autopsy or after exhumation.

The current view within the fields of radiation protection and risk assessment is that doses should be evaluated for those tissues from which tumors actually arise. Thus, in radium-exposed persons, the dose to cells lining bone, and to epithelium of mastoid air cells and paranasal sinuses is important. Our current studies of the tissue dosimetry of ^{224}Ra (a member of the decay chain following ^{228}Ra) show that previous assumptions have produced dose estimates that have been high by a factor of about three; thus, the risk coefficient used in risk assessments is low by an identical factor. Measurements on bone from persons containing ^{226}Ra have shown that it tends to accumulate in locations near the target cells; thus, actual doses delivered have exceeded the doses calculated on the basis of currently used assumptions.

Three findings from our dosimetric studies during the year all support the current view that bone cancer risk is a nonlinear function of radium intake, and two suggest that the curve is more nonlinear than we had previously thought. Analysis of radium retention data has shown that the elimination rate increases with diminishing body content, suggesting strongly that persons we had classified as "low-dose" cases may have ingested more radium and accumulated more dose than we had previously thought. When this relationship is incorporated into the analysis, it will increase the nonlinearity of the curves. Another finding that will increase the nonlinearity is the discovery of residual ^{228}Ra from dial paint in the bones of some persons who worked in Illinois factories. This ^{228}Ra will increase the skeletal dose by a factor of two for this group of persons relative to others, thus changing the dose response to a more curvilinear form. The third factor is the high radium content found in three previously unmeasured persons who died of radium-related tumors and who were exhumed for examination during the year. In one of these persons with a sarcoma, the estimated systemic intake of 32 MBq (860 μCi) was in line with the intakes that led to bone cancers in other dial workers [range 3.7 MBq to 370 MBq (100 μCi to 10 mCi)]. The fact that all of these people fell into the high-dose group does not diminish the nonlinearity of the dose-response curve.

That dosimetric issues continue to be important for radium studies is shown by the occurrence of a mastoid tumor in a 58-year-old man who had received radium as a seven-year-old child. According to calculation based on assumptions about radium metabolism in persons exposed as adults, the skeletal dose for this man would be 0.85 Gy (85 rad), less than one-eighth the dose at which similar tumors had been observed previously. Could this value be an error resulting from the misapplication of dosimetry for adults to a case of childhood exposure? A careful examination of age-dependent

models of radium retention and the observations of radium retention in the individual leads us to think not. The models predict a dose during the 28 years we studied this man before his death that substantially exceeds the dose calculated from our measurements over that period. We therefore believe it to be very unlikely that the lifetime dose is at the higher level predicted by the models.

At a given dose, latencies are much longer for head carcinomas than for sarcomas. Preliminary results of log-linear dose-response regression modeling of head carcinomas suggest nonlinearity in the dose response. For bone sarcomas, the downturn in incidence at very high doses so lacks robustness that we conclude there is no evidence for it.

An explanation is suggested for the lack of head carcinomas in the ^{228}Ra subjects: in the decade preceding 1945, several of these subjects died of brain abscess secondary to sinus/mastoid inflammation and infection. We hypothesize that in the preantibiotic era, only a small percentage of head carcinomas would have been expressed.

Radiochemical determinations of ^{228}Ra were completed on all bone samples available. Forty-eight samples of luminous paint, used by U.S. Radium Corporation from 1917-1928, were obtained through the New Jersey State Health Department. Analysis results indicate that the radium used was typical of "technical mesothorium" for that period.

Etiology of Soft-Tissue Tumors.

Although absorbed doses from gamma rays for the radium dial workers in the post-1950 period appear to have been about 20 mGy (2 rad) per year to the whole body, they have been estimated at ~80 mGy (~8 rad) per year in 1930, and up to 200 mGy (20 rad) per year in some places and at some times. Radon daughter exposures of three Working Level Months (WLM)* per year are implied by 1930 measurements. Levels only 20-40% of those in 1930 have been reported since.

Red marrow dose (50 year) from the radioactivity in bones in females was estimated at 0.2 Gy per kBq (740 rad per μCi) ^{226}Ra systemic intake, to be added to the dose (~0.64 mGy, or 64 mrad) from ^{226}Ra in the marrow; doses from ^{228}Ra are approximately twice those from ^{226}Ra. The dose is about 140% of that in males. The internal beta-particle absorbed dose is about one-quarter of the dose from alpha-particle irradiation of marrow. Gamma-ray dose from internal radium is negligible compared with the external gamma-ray dose received in the workplace.

Analyses of ^{226}Ra and ^{228}Ra in samples from a recently deceased subject containing high levels of radium were completed, and the results are now being applied to dosimetry estimates. Measurements of ^{210}Po/^{210}Pb in these samples are in progress.

*One Working Level (WL) is that concentration of the short-lived daughter-products of ^{222}Rn in any combination that has a potential alpha-particle energy of 1.3×10^5 MeV per litre of air. Exposure to 1 WL for a working month of 170 h is an exposure of 1 WLM.

No association was found between internal radium and cancers of the colon, rectum, stomach, liver, and uterus. Breast cancer is associated with high radium burdens, but the relationship is not causal. No overall excess of leukemia exists in female dial workers; two cases of myeloid leukemia did appear significantly early, although external radiation exposures could have been responsible. Leukemia, if an outcome of internal radium exposure at all, occurs much less frequently than bone sarcoma. An excess of lung cancer has been observed in the female subjects studied; whether this excess is due to smoking habits or radon-daughter exposures is being ascertained under funding from the U.S. Nuclear Regulatory Commission (NRC). Efforts are directed toward two ends: the definition of the smoking habits of the dial workers to determine whether a true excess of lung cancer exists and the search for radon measurements in workplaces and descriptions of workplaces that will enable us to estimate ventilation characteristics. In 1984, we developed computer codes for tobacco smoking, other occupational respiratory exposures, and several other variables and began to code the data from our files. Also in 1984, we acquired a large number of radon measurements for radium workplaces from the post-1950 period, which were uncovered in archives at Fordham University.

A threefold excess of multiple myeloma is more closely associated with external gamma exposure than with radium intake. Spinal cord tumors may be a minor (n = 2) consequence of radium intake.

Two erythroleukemias (a significant excess) have occurred in male radium workers exposed to both gamma and alpha irradiation; similar excesses are seen in men injected with Thorotrast (a colloidal suspension of thorium dioxide formerly used as a radiological contrast medium for the soft tissues) and in male beagles chronically exposed to gamma radiation at Argonne.

Follow-up of the Bloomsburg, Pennsylvania, radium dial workers was completed. More than 97% of ~970 workers potentially exposed to radium in the Bloomsburg plant, plus ~700 worker controls, were located. Field measurements taken with a mobile body counter were completed on 225 subjects. No residual radium from occupational exposures was detected, but observable thoracic radon daughters were observed in one-third of this population due to high domestic levels of radon and radon daughters.

Radium Metabolism and Normal Body Burdens.

An analysis of radium retention data on 13 Ottawa, Illinois, women was completed; high radium contents are associated with protracted retention of radium, putatively due to deleterious effects on bone metabolism. Preliminary analyses of radium retention data in approximately 40 Ottawa women suggest that physiological factors may also affect radium retention. Low weight for height and cigarette smoking are both correlated with a retarded radium elimination. Age at menopause was not clearly related to radium retention.

The levels of ^{226}Ra and ^{228}Ra in the drinking water of approximately 1100 communities, mostly in northern Illinois and Iowa, have been reported. A rapid method for measuring ^{224}Ra, ^{226}Ra, and ^{228}Ra in water supply samples has been developed and made available to northern Illinois communities where a potential public health problem exists.

Reproductive Effects of Radium.

A review of the records determined that data were probably adequate for analyses of full-term reproductive events and of vital status of offspring through maturity. A literature review failed to identify any known feedback loop from bone damage to estrogen or other sex-hormone levels, despite the well-known effects of estrogen levels on bone density. Hormonal effects could also be mediated through pituitary pathology.

Observations concerning the timing of live births were made incidentally to the analysis of radium retention in ~40 measurably burdened Ottawa women and suggest that the deficit in births in the women with high concentrations of retained radium occurs at later ages (27-40 years), not immediately after exposure. This finding is consistent with an ova-depletion hypothesis for reproductive effects.

The episode of the radium dial painters is certainly the best known in the history of American occupational medicine. Detailed scientific and historical synthesis of the study from its inception in the 1920s to the present, when the emphasis is on radiation risk assessment, is now under way.

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6. HEALTH EFFECTS OF INDUSTRIAL EXPOSURE TO THORIUM

J. Rundo, J. H. Stebbings, and R. E. Toohey,
Principal Investigators
H. F. Lucas, G. A. Anast, M. A. Essling,
T. J. Kotek, and F. Markun

The objective of this project is to determine the health effects of industrial exposure to thorium and the long-term pattern of deposition of inhaled thorium and its daughter products in human tissues. Knowledge of the effects and metabolism of thorium is important because of its production and potential use in nuclear fuel cycles and the need to assess the health risks of the decommissioning operations at sites where thorium was processed. Inhaled thorium, an insoluble alpha-particle emitter, is an analogue of plutonium, and inhaled thoron daughters are analogues of radon daughters.

Health end points we are studying include cause-specific mortality, with the emphasis upon cancer mortality; morbidity as determined by medical examination of living workers; and changes in physiological and biochemical parameters suggesting adverse effects on bone marrow, liver, lung, and other organs.

Long-lived thorium daughter products have been measured in living workers, and thorium levels will be determined in tissues obtained at autopsy. These measurements can be generalized to other occupational or environmental inhalation exposures to thorium and will allow quantification of the radiation doses associated with possible health effects.

We obtained the study populations from employment records of the Lindsay Chemical Company and its corporate successors. The company was primarily engaged in the extraction of thorium and rare earth chemicals from monazite ores and in the production of thorium-impregnated mantles for gas lamps. From all available records, which date back to 1925, we have identified 3542 male and 1040 female employees, but records prior to 1940 are incomplete. Our studies are primarily of 3200 men who worked at a plant site in West Chicago, Illinois, from 1940 until the plant was closed in 1973. Included among these men are those employed after the plant was merged into the American Potash and Chemical Corporation in 1958 and into the Kerr-McGee Chemical Corporation in 1967. Follow-up of the female employees (mostly mantle workers) is not planned at present.

Complete records of personal dosimeter readings of external radiation have been obtained for the period

1956-1973, that is, from the start of radiation monitoring at the plant until production ended. A total of 1236 employees were identified as wearing film badges for radiation monitoring; about 10% of the identified employees had accumulated exposures of 5 rem (50 mSv) or greater.

The results of gamma-ray measurements on a total of 312 thorium workers showed that 43 had ^{212}Bi contents greater than twice the standard error (S.E.) of the estimate (2 S.E.s are equivalent to 15 Bq) and 61 had contents greater than one standard error. Radioactivity was detectable only in the thorax region. Of the 312 workers measured, 309 had measurable amounts of thoron in exhaled breath; of these, 200 exceeded 75 mBq of emanating ^{224}Ra, indicating industrial exposure.

Computerized least-squares analyses of gamma-ray spectra obtained from measurements in vivo were extended to a total of 137 cases to estimate the relative amounts of thorium daughter products retained in the lungs. Ratios of ^{224}Ra to ^{228}Ac ranged from 0.52±0.32 to 2.0±1.2 with a weighted mean and standard deviation of 0.83±0.04. No ratios were observed with values exceeding 1.0 by more than one standard error.

Liver function was assessed through measurement of serum biochemical constituents. Preliminary conclusions are that serum concentrations of gamma-glutamyl transferase and choline esterase are not associated with the body contents of thorium daughters. Analysis of data on alanine aminotransferase is in progress.

A specific protocol was developed to evaluate pulmonary effects in 294 male former thorium workers who were examined at Argonne. The occupational history was computer coded according to any work involving possible exposure to a substance known to be harmful when inhaled. The following information was coded for smoking: age when smoking began, years smoked, and the average number of cigarettes smoked per day. The type of work done by the worker at the thorium plant was secured both from the history and from employment records. Chest x-rays were read according to the 1980 revised International Labor Organization code for pneumoconioses by three independent readers. Pulmonary function tests included forced vital capacity, forced expiratory volume, forced expiratory volume in the first 0.25 second, peak flow, maximum voluntary ventilation, and midexpiratory flow rate.

Thorium workers with body burdens (primarily thoracic) above 25 Bq ^{224}Ra equivalent were shown to exhale about 15% of endogenously produced thoron, compared to 5% exhaled by subjects with body burdens in the 15-25 Bq ^{224}Ra range. Whether by chance or in response to symptoms, highly burdened subjects were likely to have stopped smoking after thorium exposure began, inducing a false negative correlation between average adult daily cigarette consumption and thoron exhalation. White blood cell counts about 85% of expected were observed in seven subjects exhaling ≥100 pCi of thoron above predicted; no other variable examined showed a clear pattern of association. Whether these differences in fractional thoron exhalation result from different distributions of thorium minerals in the lungs of low and high burden subjects, or have a biological explanation, is not

known at present. Currently, estimates of thorium body burdens in the general population from exhaled thoron cannot be based on thoron retention fractions measured in subjects with high body burdens of thorium.

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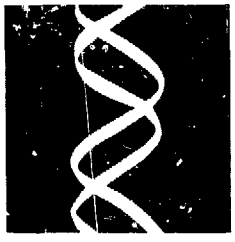

7. RADON IN HOUSES

R. E. Toohey, Principal Investigator
M. A. Essling, and F. Markun

The purpose of this study is to determine the concentrations of ^{222}Rn and its short-lived daughter products in the air of single-family houses in the midwestern U.S. This naturally occurring radioactive gas is responsible for the largest component of internal dose received by the public from background radiation. Lung cancer deaths in the United States from environmental radon, extrapolated from studies of uranium and other hard-rock miners, have been estimated at 10,000 per year. However, the magnitude of the population exposure to radon remains largely unknown. Factors that are expected to control the indoor radon level, such as soil permeability, ventilation rates, etc., are being investigated.

Radon levels in houses are determined by collecting grab samples of air in evacuated aerosol cans, which are then returned to the laboratory. The air samples are transferred into standard scintillation cells (Lucas flasks) for counting. Alternatively, time-integrating detectors (commercially available "Track-Etch"® detectors, Type SF) are placed in a house for a period of time (several months to one year) and then collected for reading. In a few houses, continuous monitors are used for detailed real-time measurements of radon and radon daughters. Simultaneously, ventilation rates and aerosol concentration and particle size are determined to enable us to determine their effects on the kinetics of radon and its daughters.

During the past year, more than 200 houses were added to the study, resulting in a total of more than 400 houses. So far, results are available for 270 of these houses, equally divided between the Chicago area and Bloomsburg, Pennsylvania. A radon concentration in air of 10 pCi per litre (370 Bqm^{-3}) was established by the Surgeon General as the original criterion for remedial action for houses in Grand Junction, Colorado, which had been built on mill tailings. More than 5% of the houses we have sampled have radon levels on at least one floor which exceed 10 pCi/L; if this figure can be extrapolated to the entire country, almost two million houses would require remedial action under the Surgeon General's criterion.

Because the radiation dose to the lungs results primarily from the alpha-

emitting radon daughters, ^{218}Po and ^{214}Po, the concentrations of ^{218}Po, ^{214}Pb, and ^{214}Bi are of interest (because of its short half-life, ^{214}Po is always in equilibrium with the ^{214}Bi). The unit in which concentrations of radon daughters are expressed is the Working Level (WL), equal to any combination of radon daughters with a potential alpha energy of 1.3 x 10^5 MeV per litre of air. This value is the potential alpha energy of the daughters in equilibrium with 100 pCi of radon per litre. Consequently, an equilibrium factor, F, can be defined as 100 WL/^{222}Rn (pCi/L). It is commonly assumed that a value of 0.5 for F is a useful figure for estimating WL exposure, given the more readily measured radon level. However, our measurements in several houses under a variety of heating and cooling conditions indicate that F = 0.2 may be a more appropriate figure for indoor air, at least in houses with forced air heating systems.

We have also directly measured the plate out or deposition of radon daughters on surfaces in a house. The usual parameter for describing plate out is the deposition velocity, obtained by dividing the deposition rate (pCi m^{-2} s^{-1}) by the air concentration (pCi m^{-3}). Values obtained by other experimenters under laboratory conditions have ranged from 0.5 to 10 mm s^{-1}; our measurements in a house gave a result of 4 mm s^{-1}, in agreement with the value of 5 mm s^{-1}, also obtained in a house, by Scott [Health Phys. 45, 481 (1983)].

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8. ALPHA-PARTICLE EFFECTS ON CELLS

R. A. Schlenker, Principal Investigator
E. G. Thompson and B. G. Oltman

This program, which deals with the mutation, transformation, and survival of cells irradiated by low-energy alpha particles, was motivated by numerous reports of cancer induction in humans by alpha-emitting radioisotopes and by published projections that most lung cancer among nonsmokers is induced by alpha-particle emitters present in the air. The epidemiological data on alpha particles provide the only information on human cancer induction by high linear-energy-transfer (LET) radiation, an issue important in the fields of occupational and environmental health. A better understanding of alpha-particle effects at the cellular level may indicate whether the damage induced by alpha particles is fundamentally different from that induced by other agents.

Monolayers of cells are irradiated with narrow and broad distributions of alpha-particle energy. The latter type mimics the distribution encountered in vivo when a radioisotope is present in tissue. The former is the type used traditionally for irradiation of cells in this and other laboratories.

Mutation is studied with P_3 cells, a line derived from human teratoma cells, whose susceptibility to mutation induction at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus has been well characterized. The assay is based on the ability of mutants to grow in selective medium containing 6-thioguanine. Transformation is studied with C3H 10T1/2 cells, a mouse embryo cell line used extensively with high- and low-LET radiation. Assay depends on the loss of contact inhibition within certain types of colonies. Cell survival is measured as a normal part of the work on mutation and transformation and also to characterize differences between various irradiation protocols. Doses range from below 10 to several hundred centigray, but emphasis is placed on the lower levels.

Alpha particles with energies typical for our experiments travel 20 μm or less in low-density materials; it is, therefore, common to remove the medium from the cell layer prior to irradiation. We have found that cell survival is very sensitive to the details of the removal procedure when cells are irradiated with a broad distribution of alpha-particle energies. To avoid problems, we now grow cells on a 1.5-μm-thick mylar substrate, which transmits alpha particles and requires no removal of medium. This proce-

dure permits reproducible results to be obtained regardless of cell density or energy distribution pattern.

Mutation studies have focused on the dose range between 10 and 70 cGy, where survival varies between 20 and 80%. With alpha-particle energies narrowly distributed about 3.0 MeV, the mutation frequency is found to rise with dose, then level off. The transition to the plateau region of the dose-response curve occurs at about 40 cGy. There is a suggestion of downturn in mutation frequency at 70 cGy, but statistical precision is insufficient to permit a clear determination. A more-limited series of experiments with a broad distribution of alpha-particle energies extending from 0 to 3.6 MeV indicates the same plateau effect but a response that is five times lower when the mutation frequencies are compared with those obtained with the narrow energy distribution at equal levels of cell survival. The response plateau contrasts with the linear dose-response curve reported for mutation induction in Chinese hamster (V79) cells at doses between 20 and 150 cGy and in human fibroblasts (H19) between about 50 and 200 cGy.

Transformation studies have focused on the dose range between about 100 and 350 cGy where a relatively high transformation frequency has been observed with alpha particles narrowly distributed in energy about 5.6 MeV. The dose-response curve increases between 100 and 200 cGy then reaches a plateau at about 200 cGy, similar to that observed with neutrons delivered at high dose rates. Compared with neutrons, the transformation frequency is a factor of 5 to 10 higher, suggesting that the ability to transform strongly depends on LET.

In conclusion, it appears that mutation frequency in P_3 cells cannot be adequately predicted from studies done with other cell types. The origin of this failure is unknown, but the observations emphasize the need for comparative studies with several cell types over a comparable dose range. In addition, mutation frequency may depend substantially on the characteristics of the energy distributions, with distributions similar to those encountered in vivo giving quite different results from the distributions commonly used for studies in vitro. If this suggestion holds, then greater emphasis should be placed on the role of energy distribution and on "life-like" distributions. Finally, the observation of a large difference in the transformation frequency between alpha particles and neutrons, two types of high-LET radiation, emphasizes that both radiations require thorough, independent study.

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Low-Level Radiation

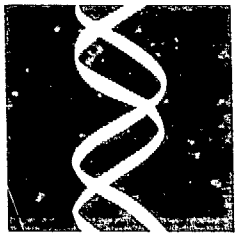
Radiation Biology and Genetics
Radiation Carcinogenesis
Radiation Hematology
Radiation Toxicology

The research conducted in the Low-Level Radiation Section is concerned with biological responses to low levels of ionizing radiation. In vivo responses such as life shortening, cancer induction, and genetic damage are studied in laboratory animals, with emphasis on the the effects of total dose, dose rate, and radiation quality. The in vivo studies are complemented by in vitro experiments with a mechanistic emphasis. In the past, primary attention was given to the development of radiation protocols, procedures, and dosimetry. Current studies, in addition, include the development of sensitive statistical approaches to enable the identification of small changes.

Life-span and genetics studies conducted by the Radiation Biology and Genetics Group and the Radiation Carcinogenesis Group of the effects of whole-body irradiation of mice have included exposure to both high and low linear-energy-transfer radiations. Recently these studies, described in Chapters 9-11, have been expanded to investigate the mechanisms and sites of damage, as well as the methods for mitigating the damage. The experimental approach has also been expanded to emphasize the biological response at the cellular level to facilitate an understanding of the responses seen in the whole animal.

Parallel life-span studies in the dog (see Chapter 12) are being conducted by the Radiation Toxicology Group to provide a stronger basis for extrapolation to man of the radiation effects that have been measured in the mouse. Species variations in radiation sensitivity, body size, and life span, among others, are being considered in the analysis and interpretation of results.

Data generated in dog studies have permitted the sequential evaluation by the Radiation Hematology Group of the response of the hematopoietic system to protracted irradiation. Because myelogenous leukemia is the earliest occurring (and one of the most common) malignancies found in man as a result of radiation exposure, this aspect of the response to low-level irradiation is particularly relevant. These studies (described in Chapter 13) emphasize the structural and functional responses of the hematopoietic system at the cellular level.



9. RADIATION CARCINOGENESIS AND RADIOPROTECTORS

D. J. Grdina, Principal Investigator
B. Nagy,* P. J. Dale, and J. M. Angerman

This program is directed at investigating mechanisms involved in radiation-induced carcinogenesis. Those radiation protocols identified in the studies described in Chapter 10 as producing significant differentials in tumor induction as a function of radiation quality will be used. Initial studies include the characterization of DNA damage and repair in the $B6CF_1$ hybrid mouse strain as these factors relate to radiation quality, exposure protocol, and specific organ sites. Selected radioprotective agents, which act in part as free radical scavengers, are being used to modulate DNA damage and repair so as to elucidate their influence, if any, on the ultimate expression of tumor induction and/or life shortening. This program is closely allied with the programs described in Chapters 1, 4, and 10.

Our studies of the mechanisms involved in radiation carcinogenesis include both in vitro and in vivo systems irradiated with 0.85 MeV fission neutrons from the Janus reactor and gamma rays from ^{60}Co sources. Radioprotectors currently being evaluated for their ability to modulate cell killing, mutagenesis, transformation, and carcinogenesis include S-2-(3-aminopropylamino) ethyl phosphorothioic acid [WR-2721], N-(2-mercaptoethyl)-1,3-diaminopropane [WR-1065] and S-3-(3-methylaminopropylamino) propyl phosphorothioic acid [WR-151327]. In vitro systems include a Chinese hamster V-79 cell system for mutagenesis studies and a C3H 10T1/2 cell system for transformation studies. The $B6CF_1$ (F_1 hybrid of the BALB/c and C57BL/6 mouse strains) and the C_3H mouse strains are being used to study tumor induction, life shortening, and acute responses as measured by 50% lethality at 6 and 30 days (i.e., $LD_{50(6)}$ and $LD_{50(30)}$, respectively) following irradiation with and without protectors. The effect of radioprotectors on radiation- and/or diethylnitrosamine (DEN)-induced hepatocarcinogenesis is also being investigated in newborn Sprague-Dawley rats. DNA damage and repair kinetics are being characterized in all systems through an alkaline elution technique. The results obtained from

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experiments in progress are summarized as follows:

1. The effects of WR-1065 (4 m$\underline{M}$) on cell survival and mutagenesis of V-79 cells following exposure to various doses of ^{60}Co gamma rays or cis-dichlorodiammineplatinum (II) (cis-DDP) have been studied. WR-1065 protected against radiation-induced cell killing only if it was present during irradiation, i.e., a dose modification factor (DMF) of 1.9. In contrast, this agent was effective in reducing radiation-induced mutations regardless of when it was administered. Following a dose of 1000 cGy of ^{60}Co gamma rays, the mutation frequencies observed per 10^6 survivors were 77±8, 27±6, 42±7, and 42±7 for radiation only, and WR-1065 present during, immediately after, or 3 h after irradiation. Protection against the cytotoxic effects of cis-DDP was evident under all conditions tested. DMFs of 2.9, 1.4, and 1.4 were obtained when WR-1065 was added prior to, during, or immediately following cis-DDP treatment, respectively. The induction of mutants under all conditions was linear as a function of cis-DDP concentration. Mutation frequencies per microgram of cis-DDP were 25×10^{-7}, 1×10^{-7}, 5×10^{-7}, and 11×10^{-7} for protocols involving no protector present or WR-1065 added before, during, or after cis-DDP treatment, respectively.

2. The ability of WR-1065 (1 m$\underline{M}$) to protect against cell killing and neoplastic transformation of C3H 10T1/2 cells exposed to ^{60}Co gamma rays at a dose rate of 50 cGy/min was further characterized in collaboration with Colin Hill (see Chapter 4). At a concentration of 1 m$\underline{M}$, WR-1065 did not protect against cell killing. The transformation frequency per cell exposed was reduced at all radiation dose levels (i.e., 200 to 900 cGy) when WR-1065 was present during irradiation. Maximum induction of transformation occurred at 500 cGy (i.e., transformation frequencies of 1.3×10^{-3} and 3.5×10^{-4} for irradiation only and irradiation plus protector, respectively). At a concentration of 4 m$\underline{M}$, WR-1065 significantly protected against cell killing, giving rise to a DMF of 1.4.

3. The agents WR-2721 and WR-151327 are being evaluated in collaboration with Carl Peraino (see Chapter 1) with respect to their protective effects against the induction in newborn Sprague-Dawley rats of enzymatically altered liver foci by ^{60}Co gamma rays, 0.85 MeV Janus neutrons, and/or DEN. Radiation induced foci in both female and male rats, with the effect being significantly higher in female animals. WR-2721 reduced the frequencies of radiation-induced foci at doses of 150 and 300 cGy. This protective effect was more readily apparent in female animals, i.e., a factor of 10 for a selected class of foci phenotypes.

4. WR-1065 has been characterized with respect to its effect(s) on radiation-induced DNA damage and repair. When present during irradiation at a concentration of 4 m$\underline{M}$, WR-1065 effectively inhibited the formation of single-strand breaks in DNA as measured by the method of alkaline elution. DNA repair was, however, perturbed by the presence of this protector. The rate of repair appeared to be slowed by about a factor of three. In contrast to results obtained with matched controls in which repair of single-strand breaks

was complete by 90 min, at least 20% of the lesions remained unrepaired up to 3 h following irradiation when cells were exposed to WR-1065. The significance of these findings is not yet clear because, under these conditions, survival of WR-1065-treated cells is enhanced while the associated mutation frequency is reduced. It may be that the radioprotector is in some way differentially affecting error-free and error-prone repair systems.

5. WR-2721 and WR-151327 are being evaluated with respect to protection against ^{60}Co gamma-ray and Janus-neutron-induced carcinogenesis and life shortening in $B6CF_1$ hybrid mice. This study, designated JM-14, uses a protocol similar to those for the J-series of experiments described in Chapter 10.

In conclusion, the radioprotectors evaluated thus far protect against radiation-induced DNA damage, cell killing, mutagenesis, and transformation. As such, they are a class of agents that can potentially be used effectively to modulate radiation damage and probe the mechanisms involved in radiation carcinogenesis. Used judiciously in conjunction with techniques such as alkaline elution to assess the magnitude of tissue-specific damage, these compounds should enable us to study effectively possible differentials with respect to the mechanisms involved in carcinogenesis induced by either fission-neutron or gamma- and x-irradiation.

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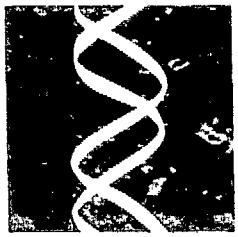

10. LIFE SHORTENING, TUMOR INDUCTION, AND TISSUE DOSE FOR FISSION-NEUTRON AND GAMMA-RAY IRRADIATIONS

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The primary focus of this program is to obtain information on the late effects of whole body exposure to low doses of a high linear-energy-transfer (LET) and a low-LET ionizing radiation in experimental animals to provide guidance for the prediction of radiation hazards to man. The information obtained takes the form of dose-response curves for life shortening and for the induction of numerous specific types of tumors. This program is closely allied with that described in Chapter 9, and that in Chapter 11, in which various end points are examined in studies that make use of the same radiation sources, dose rates, total doses, and mouse strains.

The animals are irradiated with fission neutrons from the Janus reactor and with ^{60}Co gamma rays, delivered as single, weekly, or duration-of-life exposures covering the range of doses and dose rates shown in Table 1. This array of exposure parameters includes one large study being performed for the U.S. Nuclear Regulatory Commission on both somatic and genetic effects of 60 equal once-weekly doses delivered at rates as low as 0.033 cGy of neutrons and 1.67 cGy of gamma rays per week.

Table 1. Exposure Types and Doses

Type of Exposure	Neutrons	Gamma Rays
Single exposures (cGy)	1-240	22.5-788
Fractionated, 2-60 fractions (cGy, total dose)	2-320	100-3820
Duration of life (cGy/weekly fraction)	0.67-2.67	7-32
Continuous (cGy/day)	--	1.3-6

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

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

Life Shortening.

During the past year, the last of the life-shortening studies was completed, with the exception of one small study involving only gamma irradiation delivered at low dose rates for five 22-h exposure days per week for 60 weeks. Because this last study will require about 18 months for completion, we have initiated a new interim analysis of all of the life-shortening data collected over the past 15 years. The motivation for the analysis is the increasing concern over the quality factor, (Q),* that should be used for neutrons in radiation protection. For fission neutrons, Q is 10 at present, relative to low-LET radiations for which Q is 1. There is some pressure to increase the value of Q to 20 or greater because of the evidence from studies here and elsewhere suggesting a high rate of response at neutron doses below 10 cGy--a rate that is significantly higher than has been observed in data at 10 cGy and above. Life shortening is an ideal end point for these quality factor considerations because it acts as an integrator of all radiation-induced injury that would be evident in a large population.

Our analysis takes the form of a weighted linear-quadratic covariance analysis that uses total dose as the basic independent variable, mean aftersurvival (MAS) as the dependent variable, and takes into account variation due to sex, exposure pattern, and the individual experiment. The response to gamma rays is as expected. The life shortening coefficient (reduction of MAS in days/cGy) is pattern-dependent, not significantly influenced by sex, and best derived from a linear equation. The coefficients are

single doses:	0.426±0.012 days/cGy
24 weekly doses:	
1/week	0.198±0.007 days/cGy
5/week	0.158±0.016 days/cGy
60 weekly doses:	
1/week	0.134±0.006 days/cGy

*The quality factor, Q, is used in radiation protection to reconcile known differences in the biological effectiveness of different ionizing radiations. A dose equivalence is generated from the product of absorbed dose and Q. Although Q does not refer to any specific biological end point, as does the experimentally determined relative biological effectiveness (RBE), it is conceptually related to RBE: the ratio of absorbed doses of different radiations that produce the same biological effect.

duration-of-life:

1/week	0.088±0.009 days/cGy

The response to neutrons is also linear but is little influenced by pattern of exposure up to about 20 cGy. Beyond 20 cGy, however, the response becomes nonlinear, concave downward. It is possible to derive common life-shortening coefficients from the combining or merging of all the data because of this initial, near-linear response. The data base can be truncated or delimited arbitrarily at any selected dose level so that a series of coefficients can be derived at successively increasing truncation doses. The statistically best estimates of the linear response coefficient are derived from the merged neutron data truncated at 10, 21, 30, and 40 cGy, with both linear and linear-quadratic equations (the D^2 term is typically negative in these analyses). The life-shortening values range between 3.6 and 4.9 days with a weighted mean of 3.80±0.67 days lost/cGy. The RBE values, therefore, range from 9±2 (3.80/.426) to 43±9 (3.80/.088). The response to single neutron doses only is somewhat higher than the response derived from all merged data: 6.34±1.39 days lost vs. 3.80 days. The difference is not significant, but the RBE for single doses might be as high as 15±3 (6.34/.426).

The data from the irradiation of P. leucopus have been analyzed and the response is much like that seen with Mus. Although the cause-of-death patterns are somewhat different for P. leucopus, most of the excess mortality is related to neoplastic disease, as seen in data from both mice and men. About 36% of the animals die from this cause in P. leucopus compared with 84% in Mus and 21% in man. Thus, life shortening is again confirmed to reflect excess deaths from neoplastic disease, which strengthens our reliance upon this parameter.

Pathology.

Gross pathology records are now available for analysis on about 35,000 animals. Microscopic evaluations have been completed on about 15,000. Initial analyses of dose-response functions are being performed on selected categories of the gross pathology data expressed in terms of age-specific cumulative net risk of death from the specified tumor. Net risk is estimated in 100-day intervals. Linear and linear-quadratic regression equations are fitted to the risk values as a function of total dose within the categories of sex, radiation quality, age, tumor type, exposure pattern, and individual experiment within exposure pattern. Analyses are also applied to the merged data from all individual experiments within an exposure pattern to give a summary of the responses to single doses compared with 24 once-weekly doses or 60 once-weekly doses. Dose-response coefficients and RBE values vary considerably among the different experimental variables. For example, the linear terms that are specifically used for all prediction and evaluation of responses to low doses present a configuration of values that vary in an orderly manner as a function of age, sex, exposure pattern, and radiation quality. The cogent facts are these:

1. Risk of tumor death per cGy increases exponentially with age to the midlife period (~900 days) then plateaus.

2. Females have slightly but consistently higher risk coefficients, but this effect does not influence RBE values significantly.

3. Dose protraction reduces the risk coefficients for gamma irradiation at all ages in a constant manner.

4. Dose protraction increases the risk coefficients for neutron irradiation but primarily at ages ≥500 days.

5. RBE values derived from the ratio of linear terms may increase with age for some tumors, but consistently increase as dose rate decreases, or dose is otherwise protracted by fractionation. In addition, RBE values are consistently higher for epithelial tissue tumors, either as a total category or as individual tumor types, than they are for all connective tissue tumors, collectively or individually. RBE values are also generally lower for the class of "all primary tumor deaths" than for life shortening or "all causes," even though, as noted, tumor mortality is the major contributor to life shortening.

Dosimetry.

During most of the year, Janus was not available for experimental animal studies because of a required change in the converter plate (the ultimate source of the fission neutrons) from a high level to a low level of enriched uranium-235.

Microdosimetric measurements were carried out in the High Flux Room before the change in the fission converter plate. A half-inch-diameter proportional counter was combined with a Tissue Equivalent (TE) low-pressure gas-flow system to ensure constancy of the measurements. Microdosimetric spectra were taken at five different positions within the room. Included among the positions were two standard locations defined as 0, 0, 100 cm and 0, 0, 169 cm (along a line from the face, ~1 m above the floor); positions 5 and 8 (two mouse frame positions), and the incubator position. Measurements were made with and without the mouse frames filled with mouse phantoms in positions 5 and 8 and with and without the incubator in the incubator position. All measurements were carried out at 0.5, 1.0, and 5.0 μm site diameter simulation and with the attenuator in both up and down positions. The frequency-mean lineal energy, $\bar{y}_F$, the dose-mean lineal energy, $\bar{y}_D$, and the relative contribution of low-LET radiation to the dose were some of the parameters determined from the measured spectra. At a site diameter of 0.5 μm these values were

$$\bar{y}_F = 26.9 \pm 5.1 \text{ keV } \mu m^{-1};$$

$$\bar{y}_D = 66.1 \pm 1.8 \text{ keV } \mu m^{-1};$$

and low-LET contribution = 3.2±1.4%.

At a site diameter of 1.0 μm these values were

$$\bar{y}_F = 10.2 \pm 5.0 \text{ keV } \mu m^{-1};$$

$$\bar{y}_D = 61.6 \pm 1.6 \text{ keV } \mu m^{-1};$$

and low-LET contribution = 4.7±1.6%.

At a site diameter of 5.0 μm these values were

$\bar{y}_F = 10.1 \pm 1.1$ keV μm^{-1};

$\bar{y}_D = 44.2 \pm 1.4$ keV μm^{-1};

and low-LET contribution = 8.5±0.8%.

An important result of this investigation was that no significant change in the microdosimetric spectra as a function of position or irradiation condition was observed in the Janus High Flux Room. In other words, a uniform radiation quality prevails throughout the exposure room.

The basic studies of life shortening and tumorigenesis are nearing completion, and it has become clear that the responses to low doses of neutrons (1 to 10 cGy) are nearly linear for most biological end points. Thus, a maximum value for the RBEs will be reached in this dose range for each end point. The RBE values are specific to the end point, being as low as two for connective tissue tumors or as high as 45 for life shortening from all causes of death. As more microscopic pathology data are accumulated, both cause of death and tumor prevalence data will be analyzed in detail to determine if neutron as compared with gamma irradiation induces disproportionate incidences of certain cellular types of tumors.

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11. MAMMALIAN GENETICS AND BIOSTATISTICS

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This program has three objectives: (1) to assess genetic hazards of single, weekly, and continuous doses of ^{60}Co gamma rays and single and weekly doses of fission neutrons to provide a basis for estimating relative biological effectiveness (RBE) of fission neutrons, (2) to develop detailed dose-response data at low doses as a basis for studying relationships between linear energy transfer (LET) and the sensitivity of various cell stages, and (3) to develop improved statistical approaches to analytical issues in chemical and radiation toxicology.

The genetic end points investigated in this program are (1) the dominant lethal mutation rate, (2) the frequency of abnormal sperm head morphology, (3) the frequency of reciprocal chromosome translocations induced in spermatogonia, and (4) the frequency of chromatin fragments (micronuclei) in the polychromatophilic erythrocytes in the bone marrow. Male hybrid $B6CF_1$ mice, 120 days old, are irradiated with fission neutrons from the Janus reactor and with gamma rays from our ^{60}Co gamma sources (see Chapter 22 for further descriptions of our radiation facilities). Breeding tests are performed with the irradiated males at select periods to obtain data on premeiotic, meiotic, and postmeiotic cell stages in male gametogenesis.

Effects from low single neutron doses have been compared with the effects of low doses of ^{60}Co gamma rays. A weekly exposure series has also been performed for additional neutron and gamma-ray comparisons at weekly increments of 0.67, 1.67, and 2.67 cGy of neutrons vs. 6.95, 17.4, and 32.0 cGy of gamma rays for periods up to one year. Males were periodically screened for accumulation of dominant lethal mutations and for the frequency of chromosome aberrations. An extensive study is under way for the Nuclear Regulatory Commission on the somatic and genetic effects of 60 once-weekly exposures to low neutron and gamma irradiations. The genetic tests evaluate the effects of 0.125, 0.35, and 0.67 cGy per week of neutrons and 5, 7.5, and 10 cGy per week of gamma rays. Genetic evaluations performed during the 60 weeks of exposure and after the exposure

sequence will be used to determine levels of residual genetic damage.

Genetic Studies.

The two independent studies described previously on the rate of dominant lethal mutations induced in male $B6CF_1$ mice by long-term, once-weekly exposures to either fission neutrons or ^{60}Co gamma rays have been completely reanalyzed to extract from the data the best estimates of the two coexistent mutation rates: the rate induced in meiotic and post-meiotic cell stages and the rate in the stem-cell population. The latter is small, cumulative, and largely attributable to the unbalanced gametes produced at the first meiotic division by the segregants from spermatocytes carrying balanced translocations. This contribution is confounded in the quantitative analysis of the augmentation of neutron-induced damage by dose protraction. An arcsin transform of the data was applied to eliminate heterogeneity of the variances. The data were reassembled in a manner to provide eight "age groups" in each experiment that were used to define a cumulative dose value to relate to the irradiated stem cell. Multiple linear regression analyses of the postimplant losses were carried out to define the mutation rate per cGy per week separately from the rate per cumulative cGy. For preimplant losses, the average number of implants (dead or alive) per female was regressed on cGy per week, cumulative cGy, and average number of corpora lutea per female. The results of these analyses confirm the existence of the augmentation of the postimplant mutation rate induced by low weekly neutron doses compared to single doses. The rate is increased by 67% over the single-dose response while the response to weekly gamma irradiation is reduced by 30% from that seen for single doses. Preimplant losses, as measured by reductions in the number of implants, do not show this augmentation effect even though the response to extremely low single doses did suggest the phenomenon would occur. The coefficients for the cumulative cGy term were not significant in the preimplant loss data, but they were of borderline significance in the postimplant mutation rate data. The observed or crude mutation rates for each weekly dose level were also adjusted downward by statistically removing the effect of cumulative cGy to the stem cell. These final adjusted values, when plotted against the five-week cumulative dose (the dose to postspermatogonial cells only), revealed an additional phenomenon. The postimplant dominant lethal mutation rate rose rapidly at doses below 3 cGy, then began to slacken before approaching a more monotonic linear rate of increase. The initial rate may be threefold greater than the rate seen at doses above 5 cGy. The neutron-induced augmentation phenomenon appears to reflect complex interactions between total dose, dose rate, cell stage, cell sensitivity to mutation and killing, and the microdosimetric factors of ionizing event frequency and magnitude.

Statistics.

The discrete nature of the measured end point (e.g., number of mutant colonies, number of micronuclei, number of live embryos, number of dead animals) is one of the unifying themes in the analysis of biological data. Statistical analyses, developed for continuous data and based on the

assumption of normality, may not be appropriate for count-type data. Thus, an important objective is to develop alternative approaches to data analysis that may be more sensitive and better describe the patterns of observed response.

A good example of discrete data requiring special analysis is in the radiation toxicity studies in dogs (described in Chapters 12 and 13). The end point is death from a specific type of tumor, which is a rare, discrete event. A number of difficulties immediately arise when time-to-death data of this type are analyzed. One complication is the staggered chronology of administration of treatment, such that treatment differences may be confounded with differential follow-up times. Surviving animals in an ongoing experiment result in differential censoring that further complicates statistical analysis. Stratified contingency table analysis is a statistical technique suited to this type of problem. A Fortran program designed for time-to-death data is now available on the IBM system; we have successfully used this program to analyze the data from the dog studies.

Another statistic that comes out of a contingency table analysis is the odds ratio, a standard statistic in epidemiology. Logistic regression, developed for the analysis of life table data, also generates the odds ratio. For the last decade, logistic regression has completely supplanted other forms of life table analysis in the field of epidemiology. We have tested a program in SAS (Statistical Analysis System) to conduct logistic regression (which is related to proportional hazards analysis and the concept of competing risk) to use in further statistical analyses of the data generated in Divisional studies.

Micronucleus assay data, where few micronuclei are found in a large sample of cells (e.g., red blood cells or polychromatic erythrocytes), presents another type of problem; the data are so sparse that they are essentially binary. If we assume that the number of micronuclei follows a Poisson distribution, it is possible to apply Poisson regression models. The resulting regression coefficients are maximum likelihood estimators that can be solved by iteratively reweighted least squares. A program that uses PROC NLIN in the SAS programming language has been developed for this purpose. Problems of this type can more easily be solved with generalized linear models (GLIM). The GLIM software package has been procured and is currently being tested on the PDP 11/44 system.

Once the contingency table, logistic regression, Poisson regression, and GLIM programs are completed, we plan to embed them within a user-friendly computer program. This program will allow the investigator to select the desired form of analysis by responding to a menu of questions at a terminal, thus facilitating statistical analysis of experimental data.

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Cytotoxic Effects of Colcemid or High Specific Activity Tritiated Thymidine on Clonogenic Cell Survival in B6CF$_1$ Mice
Cell Tiss. Kinetics

12. RADIATION TOXICITY STUDIES IN DOGS

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The basic objective of this program is to generate comprehensive data on the late effects of low doses of ionizing radiation in the dog--a large, relatively long-lived animal--to provide the necessary base for extrapolations of data from laboratory animals to man. The specific objectives are to (1) determine the influence of two major factors, daily exposure rate and total accumulated dose, (2) provide data for estimates of radiation-specific excess mortality in the dog to enable interspecies comparisons with existing rodent data and to develop a unifying concept of radiation damage, and (3) provide baseline data and specimens to characterize the pathogenesis and mechanisms of induction of leukemia, aplastic anemia, and other diseases, including late-occurring soft tissue tumors.

Protracted whole-body gamma irradiation from ^{60}Co sources is being given to young adult beagles 22 hours per day, 7 days per week at various exposure rates down to those allowing a nearly normal life span. In two separate but related studies, radiation is given either until death of the animal (0.3, 0.75, or 1.9 cGy per day) or until predetermined total doses (450, 1050, 1500, and 3000 cGy) are accumulated. The total doses are delivered at dose rates of 3.8, 7.5, 12.8, or 26.3 cGy per day to allow us to compare results with those from dogs previously irradiated until death at these same exposure rates. The dogs, maintained for their entire life span, are monitored and evaluated at regular intervals by hematologic, clinical, and pathological examinations. At death, a complete necropsy is performed. All data are entered into a computerized data base.

Responses of Adult Beagles to Continuous Exposure to ^{60}Co Gamma Rays.

We have continued to monitor both control dogs and those under continuous irradiation at 0.3, 0.75, and 1.9 cGy per day. As of May 1985, these dogs have been irradiated for periods ranging from 2299 to 3369 days after their entry into the experiment at 400 days of age. Generally, all dogs appear in good health; the irradiated dogs cannot be differentiated clinically from the controls, except for depression in the numbers of circulating blood cells and, in males, testicular atrophy and aspermia.

The deaths of an additional 20 dogs during the past year give a total of 47 decedents (44 experimental dogs out of a total of 184 and 3 controls out of 46). These 20 new deaths represent nearly half (43%) of all dogs that have died since the beginning of the study. The increased mortality is related to exposure rate as the 20 deaths include 12 at 1.9 cGy per day, six at 0.75 cGy per day, and two at 0.3 cGy per day.

The total number of deaths to date is also related to exposure rate as 54% of the dogs in the 1.9 cGy per day group have died, as compared to 24% of the 0.75 cGy per day group; 7.6% of the 0.3 cGy per day group, and 6.5% of the nonirradiated controls. No difference was found between the numbers of deaths in the lowest exposure rate group (0.3 cGy per day) and that in the control group (7.6% and 6.5%, respectively) after more than eight years of irradiation.

In the 1.9 cGy per day group, 13 of the 25 deaths are from malignancies of the hematopoietic system: seven were myelogenous leukemia or related myeloproliferative disorders (MPD), four were due to mast cell tumors (there was a fifth in a dog dying of myelogenous leukemia), and two were due to lymphoid tumors. Inasmuch as the mast cell is a form of the basophil and a derivative of the granulocyte/monocytic line of differentiation from the pluripotent hematopoietic stem cell, we can include the mast cell sarcomas among the myeloproliferative disorders even though they did not principally affect the bone marrow as in the other seven cases. Also, as indicated in earlier reports (on studies at higher dose rates), except for the mast cell tumors that are currently appearing, the myelogenous leukemias and related MPDs are the earliest malignancies to develop, occurring no later than 2000 days of exposure. That observation is still true in this study as the latest death due to myelogenous leukemia occurred at 1944 days of exposure while all of the fatal mast cell tumors occurred after 2000 days of exposure.

In addition to the 13 hematopoietic malignancies, there have been five other fatal tumors in the 1.9 cGy per day group; three of the five were mammary carcinomas and the others were a vascular sarcoma of the spleen and a carcinoma of the nasal cavity.

During the past year, we have also seen the first soft tissue malignancies among dogs irradiated at the two lower rates of 0.75 and 0.3 cGy per day. These recent tumors include lymphoid, mast cell, and pancreatic duct tumors in dogs irradiated at 0.75 cGy per day, which together with an earlier occurring MPD give a total of four malignancies in this group. There were also a mammary and a mast cell tumor in the group irradiated at 0.3 cGy per day for a total of two to date.

The fatal tumors include six mast cell tumors, eight other MPDs, and other soft tissue malignancies. To date, fatal tumors account for 49% of the deaths in the irradiated groups, whereas no fatal tumors have been found in the control groups.

Responses of Adult Beagles to Terminated Exposures to ^{60}Co Gamma Rays.

The dogs in this study still alive as of May 1985 range in age from 2779 to 4783 days. To date, 57% of the irradiated dogs and 62% of the control dogs have died. Although the data are still

incomplete, several interim conclusions are justified. Our ability to analyze the relationship between the proportion of tumors and dose data was greatly enhanced during the past year by our acquisition of a computer program written by the National Cancer Institute (see D. G. Thomas and J. J. Gart, 1983, Computers and Biomed. Res. 16, 116-126) and development of programs to accommodate its use with our computerized records systems.

The following conclusions on tumor incidence and irradiation dose and dose rate are based on the analyses we conducted using the program of Thomas and Gart:

1. Although age at the onset of irradiation of animals differs for the various groups, age does not affect the interpretation of results.

2. Results obtained from analyzing the data on the basis of aftersurvival or actual age at death are the same.

3. There is a positive trend in the proportion of dogs dying of tumors as total dose is increased at all dose rates except 12.8 cGy per day. If the results are combined for the overall rates, the positive trend is highly significant ($P<.0001$).

4. There is no evidence of dose rate effect except at a total dose of 3000 cGy; at this total dose, the trend is in the negative direction.

5. All of the significant positive trends across dose by dose rate are linear. There is no evidence of departure from linearity.

6. Because of the large difference in follow-up time between the dogs given a total dose of 450 cGy and the other group, there is not enough data available to make a meaningful analysis of the 450 cGy groups.

Results from dogs in earlier studies irradiated continuously at similar dose rates (and from dogs being irradiated at lower rates as described earlier in this summary) show that the mortality rate in dogs continuously irradiated is related to dose rate. This finding contrasts with that found for the terminated exposures where the effects are additive and influenced only by total dose. Studies of terminated exposures to mice in progress at this Laboratory suggest a dose rate effect but it is expected to be smaller than would be expected from duration-of-life exposures. These differences can be explained because with both species, mice and dogs, the maximum exposure is 400 days. In the case of the mouse, this period exceeds 40% of the total life span, but it is only 10% of the life span of a beagle.

Other interim observations (described in earlier reports) on significant life shortening from all causes and increased frequency of specific tumor types have not changed during the past year. As they become available, data will be further tested with the program of Thomas and Gart.

Because of the large numbers of fatal tumors in the spleen, a special effort was made to characterize these malignancies more fully. Specimens of 17 sarcomas of the spleen, 11 from this study and six from dogs chronically irradiated in earlier studies at this Laboratory, were submitted to the Veterinary Pathology Section,

Armed Forces Institute of Pathology. These specimens were also referred by the Veterinary Pathology Section to the Soft Tissue Section and the Immunopathology Diagnostic Section, as well as the Electron Microscopy Section, Clinical Center of the National Cancer Institute.

A consensus of the several reviewers, on the basis of light microscopy, electron microscopy, and immunochemical examinations, was that the tumors were anaplastic sarcomas composed of cells differentiating toward several cell types, including the Schwann cell, and are similar to radiation-induced sarcomas in man.

We are currently reviewing additional cases of tumors of the spleen from control and irradiated dogs and have also included hematomas of the spleen for review because of their high frequency in irradiated animals. At the present time, our data show a 10-fold increase in both sarcomas and hematomas of the spleen (most with extramedullary hematopoiesis) of dogs given any of several forms (internally deposited radionuclides and external whole-body ^{60}Co irradiation) of protracted irradiation.

In summary, these studies provide data that identify tissue sensitivities, target organs, disease processes (degenerative, neoplastic, etc.), life shortening values, and mortality rates that result from continuous and terminated exposures to whole-body radiations and relate them to various total doses and dose rates. The data from protracted exposures given at rates between 3.8 and 26.3 cGys per day show that the life shortening and numbers of fatal tumors are determined by total dose when the irradiation is terminated at total doses between 450 and 3000 cGys. The survival data and causes of death identified at continuous exposure to 0.3, 0.75, and 1.9 cGy per day will permit further testing of theoretical models of radiation damage and mortality. At the present time, the results support the conclusions derived from short-lived laboratory rodents that radiation damage at the low dose rates that are relevant to human populations are determined by total dose and are independent of dose rate.

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13. HEMATOPOIESIS UNDER CONDITIONS OF CHRONIC TOXICITY

T. M. Seed, Principal Investigator
L. V. Kaspar

The objective of this project is to elucidate the cellular processes by which chronic exposure to low daily doses of ionizing radiation induce and promote the development of either aplastic anemia or myeloid leukemia, i.e., two prominent pathological consequences of radiation exposure.

The experimental approach to the problem involves the sequential assessment of hematopoietic function of beagles under chronic whole-body gamma irradiation (1.9-12.8 cGy per 22-h day) during the preclinical phases of developing aplastic anemia and myelogenous leukemia (and related myeloproliferative disorders, MPD). Combined morphological and ultrastructural techniques are applied to assess the structural changes in hematopoietic tissue during the preclinical stages. Cell cloning assays are used to monitor vital progenitor populations of both hematopoietic and stromal cell origin. The care, clinical monitoring, and irradiation of the animals is provided by staff members from other groups in the Division.*

During 1984, we continued to elucidate the cellular mechanisms of hematopoietic recovery in beagles under gamma irradiation (1.9-12.8 cGy per day). This recovery appears to be a pivotal process in the progression of both aplastic anemia and myeloid leukemia. Major areas of effort include (1) structural studies of the sequential change in bone marrow architecture during the prerecovery period and (2) functional studies of the sequentially collected, marrow-isolated hematopoietic progenitors with respect to their radiosensitivity, cell cycle status, and repair capacities.

Structural Studies.

Using morphometric techniques, we have begun to document and quantitate radiation-dependent, time-dependent lesions of the endosteum (i.e., the functional critical cellular interface between the bone and marrow) in marrow samples of individuals progressing to aplastic anemia. In the near future, we will begin to examine the endosteal responses of the leuke-

*Service support personnel: D. V. Tolle (cytology), S. M. Cullen, (hematology); D. E. Doyle and C. Fox (data processing), T. E. Fritz, K. Duggal, and L. S. Lombard (pathology), W. G. Keenan and C. L. Wardrip (veterinary care), and G. L. Holmblad (irradiation).

mia-prone dogs. Results indicate that in the late preclinical stages of evolving marrow aplasia, the endosteum becomes somewhat inactive, indicated by marked reduction in formative and resorptive activities with corresponding increases in formerly quiescent areas. Such inactivation is temporally (perhaps causally) linked to the collapse in hematopoietic function in the aplasia-prone animals under continuous irradiation. The latter is reflected by the decline to less than 1% of normal pre-exposure levels in absolute marrow cellularity, hematopoietic and stromal cell progenitor concentrations, and specific marrow cell reserves.

Functional Studies.

Previously, we observed radiation dose- and pathology-dependent shifts in the radiosensitivity of hematopoietic progenitors under continuous exposure. Furthermore, we suggested that such shifts in radiosensitivity of vital target cells probably mediate hematopoietic recovery in the long-lived, leukemia-prone subgroup of dogs under chronic gamma irradiation (3.8 and 7.5 cGy per day). This observation has been extended to include shifts in radiosensitivity of hematopoietic progenitors isolated from biopsied marrow of neonates irradiated continuously (7.5 cGy per day) during both the gestational and postpartum periods. Analogous to the response produced in leukemia-prone adults under continuous irradiation, continuous exposure during fetal and postnatal periods resulted in acquired radioresistance by hematopoietic progenitors. In contrast, when the irradiation was terminated at birth, the progenitors assumed, postnatally, a nearly normal level of radiosensitivity. These results further support the suggestion that the acquired radioresistance of hematopoietic progenitors is not a clonally stable response, but instead is one that can be modified with various radiation protocols and treatments.

We have continued to explore the molecular and cellular bases of acquired radioresistance by hematopoietic progenitors under continuous irradiation.

DNA Damage and Repair. The inherent radiosensitivity of genomic DNA of hematopoietic progenitor-enriched marrow preparations has been examined through alkaline elution-microfluorometric methods. Results indicate that the extent of radiation-induced DNA damage (i.e., single-strand lesions) for both control and prerecovery marrow samples was virtually identical (i.e., DNA strand excision factors at 500 cGy were 20.1 and 20.7, respectively, whereas 20% less damage was recorded within the postrecovery-phase specimens obtained from leukemia-prone animals. DNA repair assays are in progress at present.

Cellular Repair and Recoverability. Complementary to the above DNA damage and repair assays, we continued to assess cellular repair capacity of hematopoietic progenitors isolated from marrow of both nonirradiated dogs and chronically irradiated dogs in various preclinical phases of progressing aplastic anemia and myeloid leukemia. Results of the "split-dose repair" assays show that both prerecovery and postrecovery phase hematopoietic progenitors of leukemia-prone dogs exhibited elevated survival ratios at the 24 h interfractional

period, whereas progenitors of aplasia-prone animals were markedly lower (e.g., survival ratios of 1.35 vs. 0.85, respectively). These observations suggest that the hematopoietic progenitors of leukemia-prone animals, in contrast to the aplasia-prone animals, have enhanced recoverability following radiation dose fractionation.

Cell-cycle Alterations. The extent of cell cycling within the committed hematopoietic stem cell compartment of the marrow has been assayed by S-phase-specific cytotoxic suiciding methods. Progenitor cell-cycle modification under continuous irradiation is significantly different for the two major subgroups of dogs, i.e., the radiosensitive, aplasia-prone subgroup and the radioresistant, leukemia-prone subgroup. Prior to radiation exposure, the extent of progenitor cell cycling was significantly higher in the aplasia-prone animals when compared with either the leukemia-prone animals or the nonirradiated controls. During the initial period of exposure, these aplasia-prone animals responded by first decreasing the extent of cycling and later increasing it substantially above control levels. The leukemia-prone animals, in contrast, initially responded by progressively increasing the extent of cell cycling during the prerecovery period. Following prolonged periods of exposure, cell cycling appeared to be substantially reduced during the postrecovery phase.

Two major conclusions are drawn from our work to date: first, hematopoietic recovery during the course of chronic, low daily dose, gamma-ray exposure is a "vital" and "necessary" step, but not by itself "sufficient" for the development of myeloid leukemia; and second, hematopoietic recovery appears to be mediated by a time-dependent selection and amplification of aberrant hematopoietic progenitors that express a battery of newly acquired characteristics, including increased radioresistance, modified cell-cycle properties, and enhanced clonal growth and recovery potentials.

On the basis of these interim results, our near-term goals include (1) elucidating the molecular and cellular bases of acquired radioresistance by hematopoietic progenitors and (2) investigating possible causal relationships between acquired resistance by progenitors and subsequent leukemic transformation.

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Molecular Biology

Molecular Anatomy
Molecular Biophysics
Mutagenesis
Photobiology

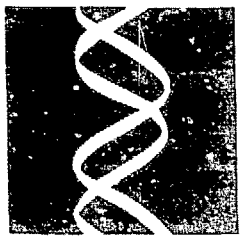
The Molecular Biology Section comprises four groups studying biological structures, biological regulation, and the modification of both structure and regulation at the molecular and cellular level. Two of the groups study the effects of mutagenic, carcinogenic, and toxic environmental radiations and other environmental pollutants on DNA and cell proteins to elucidate their modes of action. Another examines the structures of specific proteins, with emphasis on the structure-function relationship. The last group examines cell growth and its regulation, a factor intimately related to the other studies.

The Photobiology Group examines the nature of DNA damage produced by mutagenic and carcinogenic environmental radiations. They examine a variety of DNA end points such as DNA strand breakage, DNA-protein crosslinking, the formation of alkali labile sites, and the generation of DNA photoproducts, and correlate these DNA changes with cell killing and mutation. (See Chapter 14).

The Molecular Anatomy Group is concerned primarily with detecting mutation and toxic injury produced by ionizing radiation and other energy-related pollutants through the use of two-dimensional electrophoresis. They have developed methods for characterizing large numbers of cellular and extracellular proteins and are producing reference maps of the proteins of human plasma and lymphocytes, mouse liver, and other systems of interest. (See Chapter 15.)

The Molecular Biophysics Group is determining the three-dimensional structure of specific proteins, including immunoglobulins (such as anti-idiotypic antibodies and autoantibodies), diphtheria toxin, a Δ^5-3-ketosteroid isomerase, leukocyte cationic proteins, and the photosynthetic reaction center. Information is obtained by various biophysical techniques, primarily x-ray diffraction for crystalline material and gel filtration for molecules in solution. (See Chapter 16.)

Studies, described in Chapter 17, on the regulation of cell growth conducted by the Mutagenesis Group are providing evidence for similar regulatory systems in widely different types of cells, from bacteria to mammalian cells. The current approach is primarily biophysical, involving measurements of cell volume, density, and mass in synchronized cultures.



14. MOLECULAR AND CELLULAR EFFECTS OF RADIATIONS

M. J. Peak, Principal Investigator
J. G. Peak, A. Ito, and R. M. Roth*

This program is concerned with the basic nature of the biological effects of mutagenic and carcinogenic environmental radiations, including those solar ultraviolet and visible radiations responsible for the most common form of human cancer: cancer of the skin. Concentrating on the damages to DNA caused by these radiations, the program attempts to delineate the basic mechanisms whereby such damage may occur.

Since its inception, this program has studied biological effects of radiations ranging from short wavelength ultraviolet (far UV) through long wavelength UV radiation (near UV) to visible radiations. Recently, we have begun to study the effects of x-rays, ^{60}Co gamma rays, and fission-spectrum neutrons. Biological systems used as targets range from prokaryotic cells and subcellular genetically active nucleic acid to eukaryotic cells such as yeast and the human P_3 cells (see Chapters 3 and 4 for a further discussion of research involving P_3 cells). We are studying such biological end points as cell killing and induction of resistance to thioguanine and such physicochemical end points as DNA breakage (single-strand and double-strand scissions), the formation of alkali labile sites, DNA-to-protein covalent bond crosslinking, and the formation of DNA photoproducts such as pyrimidine dimers and pyrimidine adducts.

This work with nonionizing radiation has identified two principal types of pathways through which damages can occur. Far-UV radiation is toxic and mutagenic due to the direct absorption of photons because of base tautomerism, resulting in such specific photoproducts in DNA as pyrimidine dimers and pyrimidine adducts. Near-UV radiation, on the other hand, exerts its mutagenic and other deleterious effects indirectly, primarily through pathways that use nonDNA cellular sensitizers and several reactive oxygen species that are generated from ground-state molecular oxygen. These photosensitized reactions are known as photodynamic action. We are studying the details of these various mechanisms and the ultimate

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damages inflicted upon DNA, damages possibly producing the genetic changes that result in carcinogenesis. The following summarizes some of our recent results.

DNA Damages in Human Cells Caused by Nonionizing and Ionizing Radiations.

We are using the sensitive techniques of DNA alkaline filter elution to study DNA backbone scissions (frank breaks plus alkaline sensitive sites, referred to as single-strand breaks, SSBs) and DNA-to-protein crosslinks in human P_3 cells in culture. We have demonstrated that these elution techniques are an order of magnitude more sensitive than the classical sedimentation techniques we have used in the past. For example, we are now able to measure DNA damages well within the meaningful biological dose range; for example, we can detect SSBs caused by x-rays (in collaboration with Colin Hill, Chapter 4) after 0.025 Gy exposure. We are obtaining reproducible dose responses for the induction of SSBs and DNA-to-protein crosslinks by ionizing radiations of various energies, monochromatic far-UV radiation (254, 270, 290 nm), mid UV (313 nm), near UV (334, 365, and 405 nm), blue light (434 nm), and narrow band light at 470 and 490 nm, but not green light (545 nm). Near-UV and visible radiations cause many more crosslinks and SSBs per cell per lethal event than far-UV radiation. Radiation at 365 nm induces over twenty times more SSBs per lethal event than x-radiation, an observation that reemphasizes the potential for biological harm resulting from exposure to this environmentally pervasive nonionizing radiation. The biological role of these near-UV-induced SSBs and crosslinks remains an open question, and it is important to investigate the capability of normal and impaired cells to repair these damages.

We investigated the mechanisms whereby these DNA damages are induced in the DNA of mammalian cells, by the use of selected specific chemical modulating reagents. Stringent removal of oxygen during irradiation does not affect the rate of induction of DNA-to-protein crosslinks or SSBs caused by 290 nm radiation but decreases the rate of accumulation of these lesions caused by 405-nm radiation. Irradiation of the cells in a deuterated aqueous environment (compared with the normal hydrated aqueous environment) does not affect the rate of induction of these damages, whereas those induced by 405-nm radiation are enhanced in the D_2O environment. This observation is further evidence that DNA damages induced by far-UV radiation result from direct events due to absorption of photon energy by the DNA, which is chemically matched to the photon energy. Near-UV radiation effects require the presence of molecular oxygen as an essential cofactor for these pathways to proceed, implying participation of oxygen radicals and reactive species in these pathways (see Photodynamic Action, below). Since neither DNA nor oxygen are chemically matched to the photon energies of the near-UV, other endogenous chromophores (sensitizers) must operate in this region. Analyses of the relative spectral efficiencies of the induction of these DNA lesions by the wavelengths used (action spectra) support the hypothesis of indirect and direct effects and also indicate roles for bilirubin and porphyrin as sensiti-

zers in the induction by near-UV and visible radiations of SSBs and DNA-to-protein crosslinks, respectively. We have recently demonstrated that exogenously added protoporphyrin IX increases the induction of DNA-to-protein crosslinks caused by 405-nm radiation, consistent with our proposed role for its participation in the formation of this DNA damage. In conclusion, the indirect mechanisms of action of both near UV and x-rays that cause damage to DNA are similar in that they both operate via reaction oxygen species.

DNA Changes Caused by Cancer Promoters.

In a collaboration with Eliezer Huberman (Chapter 3), we have measured the effects of cancer promoters that induce differentiation of human promyelocytic leukemia cells upon DNA SSBs and DNA-to-protein crosslinks. After a lag of 1 h, the DNA of HL-60 cells exposed to 10^{-7} M phorbol 12-myristate 13-acetate (PMA, a potent promoting agent) showed an increase in both SSBs and crosslinks. The treatment induced about 100 DNA-to-protein crosslinks per cell genome, and these crosslinks persisted for at least 15 h. Weaker promoters produced fewer crosslinks, and cells that do not respond to PMA by differentiating also showed fewer DNA-to-protein crosslinks. The results indicate that DNA damages caused by promoters occur during cellular differentiation, in contradiction to the dogma that promoters operate at the level of the cell membrane. A genetic role for the induction of DNA-to-protein crosslinks has yet to be determined.

Photodynamic Action.

In collaboration with Christopher Foote, Chemistry Department, UCLA, and Norman Krinsky, Department of Biochemistry and Pharmacology, Tufts University, we are investigating the details of the chemical mechanisms whereby excitation energy is passed among photosensitizer molecules, reactive oxygen species, and DNA. We are using isolated DNA (to avoid complexities due to permeability barriers for reactants and extragenomic chemical events) and added chemical probes to attempt to identify specific sensitizers and reactive species. Several naturally occurring potential cellular photosensitizers have been identified by these methods, and roles for several of the reactive oxygen species such as singlet oxygen and the superoxide anion generated by these radiations have been postulated. We have now demonstrated that superoxide anion (O_2^-) can be generated from ground-state molecular oxygen by irradiation (270, 313, 334, 365, 405 nm, also natural solar UV) of the reduced coenzymes NADH and NADPH, thiolated nucleic acid bases, and flavin B vitamins, assayed by specific superoxide-dismutase-inhibitable reduction of cytochrome c. Thus, this anion could be generated from endogenous sensitizers within cells exposed to solar radiations. Naturally occurring metabolites chemically matched to the photons of solar UV apparently can produce O_2^- from ground-state molecular oxygen upon irradiation with solar UV. Furthermore, we demonstrated that O_2^- (produced from KO_2^-) can initiate reactions that inactivate the genetic activity of isolated transforming DNA

(50% activity destroyed by 1,500 ug KO_2^-) and break DNA in mammalian cells (1.4 SSBs/10^{10} daltons/μg KO_2^-/mL in Chinese hamster V79 cells). It is also mutagenic in Salmonella typhimurium.

A study of the photosensitized breakage of DNA in isolated transforming DNA by 2-thiouracil and 334 nm radiation is now complete. This sensitizer enhances the number of SSBs caused by the radiation 30 times at 10^{-3} M, the concentration for maximal effect. This enhancement of SSBs is inhibited by benzoate, glycerol, diazobicyclo[2.2.2]octane, histidine, and superoxide dismutase and is enhanced by irradiating the DNA in D_2O (instead of H_2O). Interpretation of these complex results is difficult and indicates that several pathways exist between the initial deposition of photon energy and the appearance of a DNA break, involving several different reactive species of oxygen, one of them O_2^-. Elucidation of further specific chemical details of the complex pathways of photosensitizations leading to DNA damages is one of our goals.

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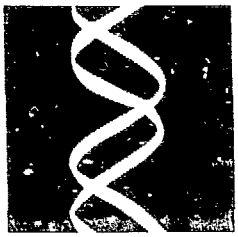
Photochem. Photobiol.



15. THE MOLECULAR ANATOMY PROGRAM

Norman G. Anderson and N. Leigh Anderson, Principal Investigators
C. S. Giometti, J. Taylor, J.-P. Hofmann,*
M. A. Gemmell, S. L. Nance, and S. L. Tollaksen

The central objective of the Molecular Anatomy Program is to develop and use high-resolution analytical techniques to detect and measure the effects of ionizing radiation and energy-related pollutants on mammalian cells and tissues. Effects produced include mutations, malignant transformation, teratogenesis, and a wide variety of types of toxic injury. The central experimental question is whether the study of these effects requires a variety of different analytical techniques, each of limited range, or whether one global analytical method can be devised that can be applied to all of these problems. We present here a summary of work supporting the conclusion that high-resolution two-dimensional (2-D) electrophoresis can be used for all these studies.

Mutations.

Various methods for systematic detection and characterization of mutations have been examined in detail, and the costs of detection by sequencing, use of restriction enzymes, specific locus testing, and 2-D protein mapping have been compared. To monitor the same number of base pairs, the relative cost of 2-D mapping is two orders of magnitude less expensive than repeated sequencing of the same DNA segments in different treated animals or cells and one order of magnitude less expensive than restriction enzyme mapping.

In addition to its lower cost, we believe 2-D mapping to be the method of choice for the following reasons. Approximately 76% of base substitutions in structural genes lead to amino acid substitutions, and approximately one-third of such amino acid substitutions produce a change in protein charge. On 2-D maps, single-charge changes can be detected quite easily over a molecular mass range of approximately 10,000 to 100,000 daltons. We have demonstrated this feature experimentally by carbamylating and mapping protein mixtures. Thus, a protein the size of actin containing 400 amino acids can reflect base substitutions in 1200 base pairs in the coding region of the corresponding gene with an overall efficiency of about 25%. Actin therefore

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serves, in effect, as a detector for a single base change in 300 base pairs. In a representative gel chosen for analysis, 716 proteins were weighted by mass and summed and found to be coded for by slightly more than 1.1×10^6 base pairs of DNA. Therefore, a single gel analysis is a detector capable of revealing any change in approximately 275,000 base pairs of coding DNA. Recent improvements suggest that this number can be increased by a factor of four, i.e., 1 megabase can be monitored by one analysis. The ISO-DALT systems for gel analysis and the TYCHO image analysis and data base systems under development aim at 10,000 analyses per year, i.e., monitoring each year a total of 10^{10} bases.

Charge shifts reflect chiefly point mutations. Therefore, it is important to determine whether other types of mutations can be detected on 2-D gels as well. Mutations involving changes in mass (additions or deletions) can be observed over the mass range of 10,000 to 100,000 daltons, providing the mass change is 2% or greater.

Detecting mutations that involve a change in the amount of gene product produced requires accurate quantitation of each of the hundreds of proteins seen on individual gels. In deletions involving total absence of expression of one allele, a 50% decrease in the amount of gene product would be expected, while with mutations affecting control genes or the genes for proteins involved in protein synthesis, smaller increments or decrements may occur. The precise and reproducible quantitation required to study mutations in control genes represents our greatest technical challenge. With the present system, 50% decrements in expression can be detected routinely, but the reproducibility is less than that required for a large-scale mouse mutation study. Additional developmental work is thus required.

Before we attempt a large-scale mouse mutation study, we must demonstrate experimentally that variants can be found, discover whether both charge and mass variants can be seen, and begin to identify new variants, i.e., to demonstrate that a wild-type protein and a candidate variant of it seen on a gel are produced by allelic genes and hence are related. We have demonstrated relatedness experimentally by showing that wild-type and variant tropomyosins coisolate through the same isolation procedure, almost always have the same mass, react with the same antisera, have similar amino acid compositions, and give similar or identical patterns when the products of partial proteolysis are mapped. Most of our model studies have been done with human muscle and nonmuscle tropomyosins. Both charge and mass variants have been found, and the relationship to the wild-type proteins has been demonstrated. In addition, we have mapped liver samples from BALBc and C57BL mice and have found several protein variants, which are currently being examined in backcrossed animals.

Our initial studies on the detection of induced mutations were done with ethylnitrosourea (ENU). In 1979, Russell and co-workers at Oak Ridge National Laboratory observed 35 mutations in 7,584 mice, or one per 217 mice in an experiment in which they used the specific locus test (seven loci) with a dose of 250 mg ENU per kg body weight in

101 X C3H F_1 mice (followed by a delay of 13 weeks before breeding). If 70 loci of equal sensitivity were tested, then the expected number of mutations would be one per 22 mice, and if about 1400 loci per mouse were examined, then approximately one per mouse might be seen. These extrapolations assume that the genes examined with the specific locus test are representative of all genes, and that charge variants are as frequent as variants that affect gene expression. Work in other laboratories suggests that these assumptions are not true and that the vast majority of mutations affect the level of gene expression without detectably altering the structure of the gene product. We are currently analyzing the offspring from mice in our first ENU experiment to determine whether mutations occur randomly or selectively.

In summary, the systems we have in place are the most efficient now available for mammalian mutation studies. However, the image analysis and data reduction systems cannot keep pace with the planned gel output and thus will require much additional improvement and modification.

From classical studies on mutagenesis in corn, Drosophila sp., and mice, it is known that large differences exist in the number of mutations observed at different loci in response to the same radiation or mutagen dose. This finding has been ascribed to differences in sensitivity of different genes (hot spots), to differences in efficiency of repair of different genes, and to differences in the importance of different genes or gene products to survival. Mutations to proteins involved in cellular structures are generally considered to be more often lethal than are mutations to proteins that remain in solution (plasma or cytosol proteins). In addition, we have proposed that mutations to proteins that are involved in differentiation are less likely to be lethal than are mutations to so-called housekeeping proteins. To settle this issue, we need to determine the intracellular location of each protein found in our maps, to determine which are base set or constitutive proteins, and to determine which proteins are associated with differentiation. This process requires high-resolution cell fractionation, careful mapping of a variety of different cell types from one species, and the development of systematic methods for identifying known enzymes and other known proteins in our maps.

The final analysis of a chemical- or radiation-induced mutation involves sequencing both the variant and wild-type genes. Specific probes are required to isolate these genes. We have developed methods for isolating enough protein from spots on gels to allow us to begin to construct specific DNA probes. The full circle of mutagenesis studies must involve the identification and sequencing of mutant and wild-type genes, identification of the gene products, and exploration of the effects of the mutation at both the cellular and organismal levels. The techniques we have developed allow this full circle to be explored.

It is important to demonstrate that deletions yielding mass changes can indeed be seen on 2-D gels. Present data suggest that during evolution the ratio of amino acid substitutions to gap events (mass changes) is about 1:42 and that the majority of deletions or additions involve less than two amino acids. It is thought that large gap

events occur regularly but are either lethal or selectively disadvantageous and therefore rarely survive to be seen. We can test this hypothesis by looking for mass variant alleles in genes whose products serve no critical biological function. Since genes for the endosperm or storage proteins of hexaploid wheat exist in multiple copies, and since these proteins serve only to store amino acids, major compositional changes should survive and be expressed. We have mapped the storage proteins of related strains of wheat and have found that mass variants occur almost as frequently as charge variants, suggesting that large numbers of deletions occur but are not ordinarily expressed. This finding suggests that wheat storage proteins may provide an excellent model for studying radiation mutagenesis unmodified by considerations of survival or selection.

Toxicology.

The action of toxic agents that are not genotoxic may involve binding to specific receptors, nonspecific covalent binding, effects on lipids that may yield a range of secondary effects, changes in gene expression, and leakage of proteins from cells and tissues. The net effect may be a change in cellular protein composition. Using 2-D mapping combined with quantitation, we determine whether a range of drugs and toxic agents produce one nonspecific cellular alteration or whether changes are seen that serve as characteristic fingerprints of classes of toxic materials. Our initial studies on human lymphocytes demonstrated that many substances (e.g., interferon) and physical insults (e.g., heat shock) produce highly characteristic results. Among the proteins turned on by interferon, we discovered a mitochondrial protein that may be the cause of interferon fever. We have now extended this work to intact mice and have found a series of unique alterations produced by Araclor 1254 treatment. A systematic series of studies is in progress in which we are using a variety of drugs and toxic agents in intact mice to determine whether characteristic responses occur and, if so, how they are related to specific intracellular events and processes. Drugs and toxic agents have been reported that stimulate production of endoplasmic reticulum, inhibit mitochondrial protein processing, cause marked enlargement of nucleoli, stimulate excess production of peroxisomes, or cause either enlargement or lysis of lysosomes. The aim of current cell fractionation studies on mouse liver is to discover the subcellular locations of each of the major proteins seen in 2-D maps and to discover whether induced changes involve all of the proteins of a specific organelle or only some of them. Results to date support the view that toxicology can now be largely rewritten in terms of specific cell responses and cell control circuits.

Basic Mapping Studies.

Master reference maps of human plasma proteins that include more than 300 proteins have been prepared and published. Maps of human urinary proteins, proteins of various strains of Drosophila sp., the seed proteins of wheat and rice, and the tissue proteins of corn are in preparation.

Technical Developments.

Our studies to date demonstrate the range of studies that involve the high-resolution 2-D systems we have developed. Orienting studies have demonstrated that large increases in resolution and reproducibility are possible with further development and that much higher throughput will be possible with automation, application of robotics, and improvements in scanners, image processing software, and computer hardware. Arrangements are in progress to transfer further developmental work to industry.

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16. STRUCTURAL STUDIES OF PROTEINS

M. Schiffer, Principal Investigator
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T. S. Young, C. F. Ainsworth, and F. A. Westholm

This program is directed toward the ultimate goal of elucidating the molecular events underlying selected biological processes. As a means to this end, we are involved in a variety of studies aimed at developing an understanding of fundamental structural and conformational relationships for key biological molecules. Molecules currently under investigation include immunoglobulins, diphtheria toxin, Δ^5-3-ketosteroid isomerase, leukocyte cationic protein, and the photosynthetic reaction center. Information is obtained by various biophysical techniques, primarily x-ray diffraction for crystalline material and gel filtration for molecules in solution.

Immunoglobulins.

Structural Studies. We are studying the detailed three-dimensional structure of several Bence-Jones proteins (immunoglobulin light chains) by x-ray diffraction. These proteins are excellent models for intact antibody molecules and other representatives of the immunoglobulin superfamily. The investigation is providing information on the relative positions of the immunoglobulin domains and revealing the molecular basis of interdomain interactions, thus greatly increasing our understanding of the antibody binding site.

We continued the determination of the structure of the λ_I-type Bence-Jones protein Loc. We further refined the structure at 3 Å resolution and collected data to 2.5 Å resolution (45,000 reflections) with a position-sensitive detector at the Biotechnology Resource at the University of Virginia. The Loc structure is significant because it shows a new, previously unobserved, strategy of interaction of the variable domains. The Loc structure may represent not only a different form of the antigen binding fragment (Fab) but also an anti-idiotypic antibody or T-cell receptor. Anti-idiotypic antibodies can mimic the original antigens in their biological actions. The protruding third hypervariable residues on the Loc structure could be responsible for this mimicry. In addition, the two grooves formed by the Loc variable domains can be considered as two independent binding sites. The amino acid

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sequence of the T-cell receptor was determined recently; it consists of two chains that have extensive sequence homology with the immunoglobulin light chains. A Loc-like structure might be a good model for a T-cell receptor, which can bind two molecules simultaneously.

We are also studying anti-idiotypic antibodies and autoantibodies, which are antibodies with special binding characteristics against other antibodies. Anti-idiotypic antibodies constitute a part of the network that regulates the immune response. Rheumatoid factors, a class of autoantibodies, are antibodies against the most common antibody molecule, IgG, and have been observed in a number of clinical conditions. To understand the function of these antibodies in terms of their three-dimensional structure, we are determining their binding properties in solution and are attempting to crystallize them.

During this year, we developed the procedure for purifying IgM rheumatoid factors Riv and Bor from the plasma and for preparing the variable portion (Fv) of the antigen-binding fragment from Riv and the antigen-binding fragment from the Bor protein. We are carrying out crystallization experiments with both the free fragments of protein Sch (previously prepared), Riv, and Bor and the complex they form with the constant portion (Fc) of IgG protein.

Interaction Studies. To understand the relationship of the structure of the antibody binding site to the functional properties it expresses when binding to a protein epitope, we are developing procedures to quantify the antibody-antigen interaction. These procedures are based on changes in the chromatographic elution properties of proteins when they combine to form a complex. We have developed a model of the processes that occur during the chromatography of interacting proteins such as an antibody and antigen pair. Computer simulation based on this model allows us to estimate the association constant expressed by the antibody under the conditions of the experiment.

To efficiently obtain experimental data, we have designed a microcomputer-interfaced chromatography system for gel filtration chromatography. This system is currently capable of generating 1000 data points from a 1-h analytical run with a sensitivity sufficient to visualize approximately 100 ng of protein. Data are transferred by telephone to a PDP 11/44 computer for comparison with simulated elution profiles. We are currently refining the system to include computer control of the experimental temperature for thermodynamic studies and automated sample injection to provide an opportunity for 24-h data collection.

This methodology has been applied to the binding of an Fab derived from IgA rheumatoid factor Sch to the Fc obtained from the IgG1 myeloma protein Dri. The experimental data were found to be best fitted with an estimated association constant of approximately 1.5×10^5 $\underline{M}^{-1}$. Moreover, the data indicated that the Fc was antigenically bivalent. In another example, preliminary experiments with a monoclonal idiotype-anti-idiotype combination suggested an association constant of at least 10^7 $\underline{M}^{-1}$. As the association constant is a measure of the free energy change upon bind-

ing, it is a combination of the enthalpy and entropy changes that occur. Thus, characterization of the temperature dependence of the association constant provides a means to estimate these changes and, accordingly, separate the hydrophobic and ionic contributions to the specificity-determining properties of the binding site.

Model Building. The receptor for polymeric immunoglobulins is a member of the immunoglobulin superfamily on the basis of amino acid sequence. The ligand-binding part of the molecule is made up of five domains that are homologous to light chain variable domains. We used our detailed knowledge of the molecular structure of the variable domain, which is based on our x-ray diffraction studies, together with the extensive physical and chemical data available on the receptor IgA complex to construct a model for the complex. From the model we identified the disulfide bonds that bind the IgA to the receptor. In addition, we identified intramolecular disulfide bonds in both the IgA and the receptor molecule. The methods we have developed for this project can be used to construct models for other members of the immunoglobulin superfamily.

Other Proteins.

Diphtheria Toxin. This protein is a single polypeptide of MW 53,342 with the ability to introduce an intact fragment of itself through cell membranes. This intact fragment then acts as an enzyme within the cell to stop protein synthesis, thereby killing the cell. By determining the structure of diphtheria toxin, we can learn how an aqueous protein interacts with and inserts into membranes and how this interaction permits the transmembrane movement of an intact polypeptide. Our original toxin crystals proved intractable, because of inhomogeneity of our toxin material. Indeed, this variability in its physical properties is a direct consequence of the complex behavior of the toxin. Consequently, during the past year, we developed our own protocol for toxin purification and biochemical evaluation. We can now purify 25-50 mg each of four variant forms of the toxin, and we have been able to crystallize three of these four variant forms.

Δ^5-3-ketosteroid isomerase. This enzyme catalyzes a metabolic step necessary for synthesis of steroid hormones. It binds tightly to certain steroids that are competitive inhibitors of the enzyme; thus this enzyme can also mimic certain features of steroid receptor proteins. Steroid-protein interactions play significant roles in normal development and metabolism, as well as in numerous diseases, including cancers of the breast and prostate. Although many steroid hormone structures have been solved, no proteins involved in steroid interactions had been solved until now. We have solved the three-dimensional structure of this enzyme at 2.5 Å nominal resolution during this past year; in particular, we determined the locations of its 125 amino acid residues. Moreover, we developed an interpretation of the active site of the enzyme, which agrees well with the large body of spectroscopic, chemical, and biochemical data accumulated about this enzyme during the past 20 years. We believe we understand why the enzyme is dimeric, how it binds and modifies steroids, and how this inter-

action might relate to other less-well-characterized proteins that interact with steroids.

Leukocyte Cationic Proteins. These proteins are natural antimicrobial agents found in the phagocytic leukocytes (macrophages and neutrophils) of mammals. With only 33 or 34 amino acid residues, these peptides would lack defined rigid structures if it were not for the three covalent disulfide bonds within them holding them together. Thus, these peptides survive boiling and even autoclaving, retaining extremely wide antimicrobial activity against fungi, yeast, gram-positive and gram-negative bacteria, and some viruses, at concentrations commensurate with those of penicillin. We have good crystals of one such peptide, NP2, from rabbit neutrophils. We have collected one complete three-dimensional data set from these crystals to 2.4 Å resolution and are now preparing to analyze these data using a method pioneered by Wayne Hendrickson and others at the Naval Research Laboratory. This method depends on the anomalous dispersion of sulfur atoms and will enable us to define the structure by locating the sulfur atoms.

Photosynthetic Reaction Center. This specialized complex is an intrinsic pigment-containing membrane protein that carries out the primary events of photosynthesis. The reaction center from Rhodopseudomonas sphaeroides R-26 was crystallized in a form suitable for high-resolution structural studies; it consists of three protein subunits, four bacteriochlorophylls, two bacteriopheophytins, and two ubiquinones. Determination of the structure of the R. sphaeroides reaction center is important because this reaction center is the one most commonly used for studying bacterial photochemistry and has been extensively characterized through biochemical and physicochemical techniques. Furthermore, it is one of only three membrane proteins that have been crystallized in a form suitable for high-resolution studies. The crystals are orthorhombic $P2_12_12_1$ and have unit cell dimensions of a = 142.2 Å, b = 139.6 Å, and c = 78.7 Å. There is one reaction center (MW = 10^5) in the asymmetric unit. A complete native data-set to 3.7 Å resolution was collected with oscillation photography. A search for heavy atom derivatives is currently in progress. In collaborative research with investigators in the Chemistry Division at ANL, we will be correlating our data on the structure of this complex with their data from spectral studies.

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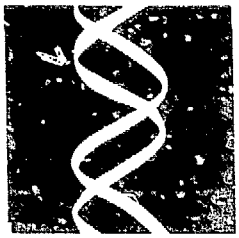

17. REGULATION OF CELL GROWTH

H. E. Kubitschek, Principal Investigator

The objective of this program is to elucidate common cellular and molecular mechanisms involved in the regulation of cell growth of both prokaryotes and eukaryotes. We are particularly interested in the kinds of regulatory mechanisms that may exist over broad evolutionary ranges of cell types as a basis for identifying major regulatory controls for cell growth during the cycle.

Our current approach is primarily biophysical, involving measurements of cell volume, density, and mass in synchronized cultures. Cells from exponentially growing cultures are separated by size, and therefore by age, by velocity sedimentation in density gradients; we usually select the smallest and youngest cohort of cells for synchronous growth. Increases in cell volume during the cell cycle are determined with a precision Coulter counter-multichannel analyzer cell-sizing system developed for these experiments. We have also developed a computer-assisted program for life-span analysis of cell growth kinetics. Values of cell buoyant density are obtained by equilibrium density centrifugation in Percoll (a suspension of silicon particles covered with polyvinyl pyrrolidone) gradients. Cell mass increase, the fundamental parameter of cell growth, is calculated as the product of cell volume and density.

Earlier we found that cell buoyant density was extremely constant during the cell cycle, and therefore, essentially independent of cell age in cultures of the bacterium Escherichia coli, the fission yeast Schizosaccharomyces pombe, and also in suspension cell cultures of three different mouse cell lines. In addition, we observed that cell buoyant density was also independent of culture growth rate as measured in E. coli and S. pombe. These findings were unexpected, and were particularly remarkable for E. coli because mean cell volumes varied by a factor of five and RNA content by a factor of eight in these experiments. We also obtained evidence that the regulatory system for cell buoyant density is osmoregulatory in nature, at least in E. coli, as cell buoyant densities were increased by the addition of either sodium chloride or glucose to the growth media. Cell growth rate and its variability were also dependent upon osmolarity.

Our observations provide evidence for similar or common regulatory systems

for buoyant density in widely divergent types of cells that divide by equatorial fission. The narrow ranges of the density distributions in individual cultures suggest that growth states are sharply defined and that critical values of buoyant density must be reached before growth can take place.

These conclusions, however, cannot be drawn for the budding yeast Saccharomyces cerevisiae. In these cells, buoyant density varied cyclically by about 2%. It may be that buoyant density constancy is a property only of cells that divide by equatorial fission.

Because cell buoyant density was constant throughout the cell cycle in E. coli and S. pombe, cell mass increase is directly proportional to cell volume increase in these cells. Therefore, our observations of constant rates of increase in cell volume during most or all of the cell cycle in E. coli, and recently in S. pombe, require that cell mass increases in the same fashion in both kinds of cells. That is, our results indicate that despite the large numbers of genes known to be involved in regulation of cell growth, mass growth rates vary little or not at all during most of the cycle and cell mass increases linearly. Such a linear increase, however, could lead to a doubling in growth rate of the mother cell shortly before cell division to allow the combined rate of mass increase of the two daughter cells to double, because, on the average, growth rates of the daughter cells must be the same as those of their mothers in steady-state cultures.

Although cell mass is the fundamental parameter of growth, earlier published information on cell mass increase is extremely sparse, as buoyant density measurements were quite difficult to perform before the advent of Percoll gradients. As discussed above, our findings of constant buoyant density and linear volume increase in E. coli and S. pombe permit us to conclude that mass growth of both kinds of cells is linear during almost all of the cell cycle. That these two very dissimilar cell lines follow the same growth law suggests that, just as for buoyant density in the two systems, growth regulation may be controlled by very similar regulatory processes conserved during evolution. Our results, of course, are currently too limited to determine the range of generality of the phenomena of constant buoyant density and growth rate. Clearly, we intend to examine growth in other kinds of cells.

In summary, we have identified two kinds of regulatory systems for cell growth, both of which appear to operate in very divergent cell types, including bacteria and eukaryotic cells. We anticipate that these regulatory systems may also operate more generally in other cell types, a possibility that will be tested. This work should provide insight into the cellular and biochemical events involved in the regulation of cell growth. Ultimate knowledge of the mechanisms providing such tightly regulated control is vital to the understanding of cell growth.

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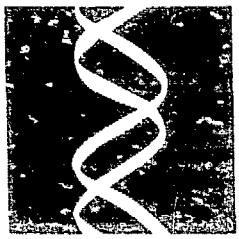
Toxicology

Chemical and Biological Characterization
Chemical Toxicology
Metal Toxicology
Neurobehavioral Chronobiology

The Toxicology Section is composed of four groups. Activities for two of them are encompassed within the Argonne Toxicology of Complex Chemical Mixtures Program. These groups, Chemical Toxicology and Chemical and Biological Characterization, conduct studies designed to determine the principles of action within energy-related complex chemical mixtures that govern the ultimate toxicity of the mixtures. The complexity of such mixtures and their almost unlimited variability make knowing these principles of action essential to credible assessments of their potential toxicity. The Chemical Toxicology Group applies a variety of in vitro and in vivo toxicologic procedures to pure chemicals, simple mixtures, and complex mixtures to determine what modifications, if any, are caused in a given toxicant by the presence of nontoxic components. Currently, primary interest is focused on metabolism of components. The Chemical and Biological Characterization Group uses sophisticated analytical chemistry techniques coupled with biological evaluations of toxicity to identify components of interest within mixtures. These studies are described in Chapters 18 and 19.

Another group, Metal Toxicology, deals with the uptake and metabolism of toxic metals in experimental animals. Interest is currently centered on the effects of such factors as age or physiological conditions (e.g., pregnancy and lactation) on the uptake and thus potential toxicity of lead, cadmium, plutonium, and other actinide elements. (See Chapter 20.)

The fourth group in the Toxicology Section, Neurobehavioral Chronobiology, studies circadian rhythms in mice and rats. Such experiments allow projections of potential deleterious effects in humans that might result from disruption of circadian rhythms, which often occurs in shift workers. Substantial efforts are also directed toward the evaluation of the effects of electric fields on circadian rhythms. This work is presented in Chapter 21.



18. TOXICOLOGY OF ENERGY-RELATED COMPLEX MIXTURES

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Recent investigations in this program and others have demonstrated that interactions among individual components in energy-related complex mixtures modify the overall toxicity of the mixtures. The toxicologic effects are usually more or less severe than those predicted by knowledge of responses to the individual components in the mixtures. Rarely are the effects of individual components simply additive. Because toxicological testing of all possible mixtures is an impossible task, it is important to understand the principles of interaction that modify responses so that credible risk predictions can be made. The Chemical Toxicology Group performs fundamental research focused on defining the mechanisms of these chemical, physical, and pharmacological interactions. Current studies include determining (1) direct toxic effects, (2) indirect effects on metabolism, and (3) the ultimate fate of toxic chemicals and mixtures. Although our investigation is directed at compounds related to energy production, our experiments are designed to be interpreted in the broader context of complex chemical mixtures from all sources. Because of the obvious need for strong chemical support for these studies, this project is closely integrated with the Chemical and Biological Characterization Group (see Chapter 19).

We take two general approaches. In one approach, we measure responses to binary mixtures, simple synthetic mixtures of chemicals, and fractions of naturally occurring complex organic mixtures in a variety of in vitro systems. The results form the basis for animal studies to examine the validity of the in vitro observations. Independent study of each of the major chemical classes of a mixture allows more sensitive and accurate estimation of the toxicity of each class of compounds and, through recombination experiments, enables us to determine the contribution of each class to the overall toxicity of the mixtures. In the second approach, we conduct whole animal experiments followed by in vitro studies designed to elucidate the mechanistic basis of observed interactive responses.

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Our in vitro studies initially focused on bacterial systems; however, current emphasis has shifted to mammalian cells. Although bacterial mutagenesis systems provide useful indications of carcinogenic potential, the use of mammalian cell systems offers some advantages because of their closer relationships with humans. Mammalian cells in culture can be reliably used for detecting mutagenic activity of single compounds, but difficulties are encountered in measuring the effects of complex mixtures. The results of our attempts to measure mutagenic activity for complex energy-related chemical mixtures in Chinese hamster ovary (CHO) cells showed that at concentrations of sample sufficient to observe minimally significant mutagenic responses, cell survival was unacceptably low (e.g., < 15%). We have examined this cytotoxicity by identifying the principal cytotoxic components, determining their basis for action, and exploring approaches whereby cytotoxicity can be preferentially diminished.

By fractionating the polycyclic aromatic hydrocarbons (PAH) isolated from a coal gasification tar, we found that the nonmutagenic 2- and 3-ring PAH were primarily responsible for the cytotoxicity of the complete PAH fraction. These compounds were only cytotoxic after metabolism by the microsomal enzymes required for activation of the mutagenic PAH. We found that the toxicity of the metabolites, hydroxy-PAH and quinones, could be markedly diminished by adding (1) uridine diphosphoglucuronic acid (enzymatic reaction with hydroxy PAH to form glucuronides) and (2) the cytosolic liver enzyme DT-diaphorase to the culture medium, which catalyzes the 2-electron reduction of quinones, thus preventing 1-electron reduction of O_2 accompanied by formation of reactive oxygen species. Experiments are in progress to determine whether the presence of these agents that diminish cytotoxicity will enhance the mutagenic response to complex, energy-related chemical mixtures.

Parallel studies of mutagenicity have led to modifications of the assay procedure to increase mutagenic responses. For example, the presence of calcium and magnesium salts enhances mutagenic responses, and responses to energy-related materials are enhanced by removing nonmutagenic phenolic compounds prior to testing. In addition, we found that useful dose-response relationships for mutagenesis are generally obtained at unusually low concentrations of hydrocarbon mixtures. The basis for these observations is being investigated, and the results are being applied to experiments designed to better understand and predict the toxicologic responses to mixtures.

The second approach, whole animal studies, is currently focused on the effects of mixtures on dermal, liver, and bladder carcinogenesis following dermal exposure. Treatment protocols include chronic exposure and initiation/promotion protocols. Dermal carcinogenesis is of concern for occupational exposure to energy-related complex mixtures, and investigation of the process in mice allows us to study interactive effects in chemical carcinogenesis. By comparing the effects of chronic, long-term dermal treatment of mice with coal gasification tar, or its neutral, basic, or acidic fractions, we first demonstrated that

the response to the neutral fraction (containing carcinogenic polycyclic aromatic hydrocarbons and other classes of aromatic compounds) was diminished by the presence of the other components. To examine the scope of this phenomenon, we are currently conducting a number of fractionation/recombination experiments. In one completed study, we separated the nonpolar (aliphatic and aromatic hydrocarbons) and more polar neutral components (see Chapter 19) and examined their ability alone or recombined to initiate tumorigenesis in a mouse dermal initiation/promotion model. The nonpolar components were more potent than the polar components. Treatment of the animals with a combination of the two fractions revealed that the weakly tumorigenic polar fraction inhibited the response to the tumorigenic nonpolar fraction. Parallel experiments are in progress in which we are using the CHO mammalian cell mutagenesis assay.

Using a hepatocarcinogenesis model in which we can distinguish initiating and promoting activities (see Chapter 1), we were able to demonstrate that the topical application of coal liquids or tars resulted in the promotion of preinitiated hepatocytes. This study, performed in collaboration with Pacific Northwest Laboratory, suggests that the hepatic response was produced by promoters in the mixtures that were absorbed and subsequently transported to the liver. This interpretation is supported by studies in our laboratories and others documenting the percutaneous absorption of topically applied coal tar, heavy distillate, and constituents from the tar and distillates, and the subsequent rapid induction of hepatic enzymes, including microsomal monooxygenases and ornithine decarboxylase. Experiments are in progress to (1) detect tumor promotion in addition to promotion of preneoplastic foci, (2) identify the promoting agents in the coal-derived mixtures, (3) examine the promoting activities of other agents and environmental mixtures in this system, and (4) elucidate the mode of action of the promoters.

In conclusion, we are examining interactive effects that modify the toxicity of components present in mixtures. Discovery of general principles of action will allow more credible risk assessment of exposure to toxic mixtures.

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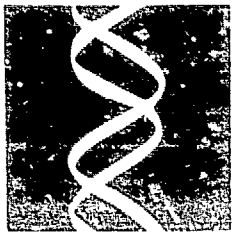

19. CHEMICAL CHARACTERIZATION AND METABOLISM OF CARCINOGENS

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The studies reported here are part of a multidisciplinary effort designed to examine the health risks involved in exposure of humans to complex, energy-related mixtures of chemicals. The specific objectives of this component of the program are to (1) chemically characterize energy-related chemical mixtures, (2) develop and use improved procedures for isolation and analyses of specific components of these complex chemical mixtures, and (3) study the role of metabolism on the toxicity of energy-related chemical carcinogens, with current emphasis on aromatic amines and aromatic hydrocarbons. Efforts addressing these objectives are closely linked because chemical procedures are developed and improved in response to needs in the toxicologic research.

Instrumental methods include high-performance liquid chromatography (HPLC), high-resolution, capillary column gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS). Analytical HPLC is used to identify and quantitate individual compounds or to distinguish groups of compounds. Semipreparative (milligram) and preparative (gram) scale HPLC is used to provide sufficient material either for subsequent fractionation or toxicologic assays. Other techniques include fractionation by liquid-liquid partitioning and open column chromatography, and spectrophotometric analysis including Fourier-transform infrared (FTIR) spectrometry for detection in gas chromatography.

We have used a series of HPLC techniques, each separating compounds on the basis of different physicochemical properties, to fractionate mutagenic coal conversion materials. An iterative approach was used whereby decisions concerning the fractionation routes were based both on toxicologic significance and chemical analysis (GC and GC-MS) of HPLC fractions.

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We devised an HPLC procedure for a separation of aromatic bases (aromatic amines and azaarenes) present in energy-related materials, and identified 3-ring aromatic amines as major contributors to the mutagenic activity of energy-related materials. This observation led to studies of the metabolism of aromatic amines and to studies of aromatic amines as bladder carcinogens (see Chapter 18).

We used similar principles to identify numerous mutagenic polycyclic aromatic hydrocarbons (PAH), including well-known compounds (e.g., benzo[a]-pyrene) and others for which little toxicologic information is available. Related studies at this Laboratory and elsewhere indicated that PAH were primarily responsible for dermal carcinogenesis of complex energy-related chemical mixtures. However, toxicologic data suggested directly or indirectly that the potency of PAH as dermal carcinogens was diminished in the presence of other components in the mixtures. On the basis of our understanding of the chemical composition of the mixtures and the objectives of the studies of toxicologic interactions, we used HPLC to obtain fractions of PAH, defined by their aromatic ring number, from a complex mixture (coal gasification by-product tar). The availability of these fractions, along with an understanding of their composition (as determined by GC and GC-MS), allowed studies of the cytotoxicity, mutagenicity, and toxicologic interactions of PAH in cellular systems (see Chapter 18). Parallel efforts involved separation and characterization of acidic, basic, and neutral fractions, as well as subclasses of neutral components (fraction S-1 containing aliphatic and aromatic hydrocarbons and S-2 containing primarily more polar heteroatomic aromatic compounds) for use in studies of interactions in the induction of bladder carcinogenesis, dermal carcinogenesis, and mutagenesis in mammalian cell cultures.

Because the results of toxicologic experiments indicated that weakly carcinogenic polar neutral components decrease the potency of coadministered carcinogenic PAH (see Chapter 18), we have characterized this group of compounds, which includes indoles, carbazoles, and keto compounds, the latter selectively detected using FTIR as a GC detector. A qualitatively and quantitatively similar array of chemicals was found in tars from two coal gasifiers, suggesting general applicability of the observations.

Metabolism of Carcinogenic Polycyclic Aromatic Amines.

Energy-related complex mixtures contain carcinogenic polycylic aromatic amines. We are studying their metabolism to better understand the dramatic differences in mutagenic and carcinogenic potency between structurally similar aromatic amines. The results will contribute to the design and interpretation of studies of the toxicologic effects of aromatic amines when present in mixtures.

We are initially examining the metabolism of 2-aminoanthracene (2-NH_2An) by rat liver microsomes. The liver is a major site of drug and carcinogen metabolism, with qualitatively similar reactions occurring in other organs. The potent mutagen and carcinogen, 2-NH_2An, which is structurally related to the human bladder carcinogen 2-aminonaphthalene, was

chosen for study because it has been shown to be one of the most mutagenic aromatic amines present in energy-related mixtures. We use HPLC to separate and quantitate metabolites generated.

Microsomal monooxygenases activate certain aromatic amines by catalyzing N-hydroxylation, while ring hydroxylation is a detoxication route. We have found that the extent of metabolic activation of 2-NH_2An via N-hydroxylation is dramatically influenced by pretreatment of animals with chemical inducers, reflecting changes in the relative amounts of different monooxygenases. Although definitive results are not yet available, we found that the isomeric 1- and 2-NH_2An are metabolized differently, especially with regard to their response to inducers.

The measurement of N-oxidation of 2-NH_2An has proven to be technically challenging. Although purified N-hydroxy-2-aminoanthracene synthesized in our laboratory is stable during storage, it undergoes nonenzymatic secondary reactions under the conditions of the metabolism and analysis procedures. We found that it (1) is oxidized to 2-nitrosoanthracene by trace metal ions associated with the HPLC system and (2) undergoes as yet unknown reactions with other metabolites. The presence of chelating agents and the reducing agent ascorbic acid minimize oxidation during HPLC analysis. We are refining approaches to minimize or control secondary reactions occurring during metabolism and during sample processing before HPLC analysis.

The results, generally applicable to the study of aromatic amine metabolism, provide insight regarding the toxicologic significance of the reactivity of N-oxidation products. Meaningful measurements of the metabolism of aromatic amines require an understanding of the chemical properties of the metabolites and the use of appropriate conditions for analysis.

In conclusion, we are actively engaged in developing and using procedures for fractionation and chemical characterization, both to better understand the properties of energy-related chemical mixtures and to contribute to toxicologic studies. In related studies, we are using chemical and biochemical approaches to examine the mechanism of metabolic activation and structure-activity relationships for aromatic amines present in energy-related mixtures.

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20. METAL METABOLISM AND TOXICITY

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This research program is designed to investigate health effects of toxic metals in animals under conditions relevant to potential human exposures. One research area focuses on the role of pregnancy and lactation in susceptibility to the toxic effects of cadmium and lead; responses under investigation include (1) lead-induced changes in pathways for vitamin D and calcium metabolism and (2) cadmium-induced alterations in kidney function and skeletal structure. The second area focuses on the gastrointestinal (GI) absorption of plutonium and other actinide elements; studies are currently being conducted in nonhuman primates to develop a procedure to determine GI absorption values of uranium and plutonium that does not require sacrifice of the animal.

For both areas of the program, elements are administered orally. Pathways for uptake and tissue deposition are studied through use of either radioactive isotopes measured by gamma spectrophotometry (^{109}Cd, ^{237}Pu) and alpha spectrometric isotope dilution (^{236}Pu, ^{238}Pu, ^{239}Pu, ^{233}U, ^{236}U), or stable isotopes measured by atomic absorption spectrophotometry (Cd, Pb). Toxic responses to lead are evaluated by competitive binding protein assays for vitamin D metabolites and by measurement of blood concentrations of protoporphyrin, calcium, lead, and hemoglobin. Toxic responses to cadmium are evaluated by analysis of urinary concentrations of cadmium, amino acids, proteins, glucose, and creatinine, and by analysis of calcium content and microradiographs of bone.

Metabolism and Toxicity of Compounds of Cadmium and Lead.

Influence of Exposure to Lead on Vitamin D Metabolite Concentrations in Young, Growing Rabbits. To study the mechanism of interaction of lead with pathways of vitamin D metabolism, we aim to identify an animal system in which serum concentrations of 1,25(OH)$_2$vitamin D decrease in

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response to lead exposure, as was reported in children with elevated levels of lead in blood (Rosen et al., 1980, N. Eng. J. Med. 302, 1128). The concentration of this metabolite, which is essential to the maintenance of calcium homeostasis during periods of calcium stress, normally increases during pregnancy and lactation and also during childhood. In our previous study with rats, we showed that during pregnancy and lactation, blood lead levels were high enough to cause lead-induced increases in heme precursors (free erythrocyte protoporphyrins, FEPs). However, no lead-induced changes occurred in circulating $1,25(OH)_2$vitamin D levels; the five-fold rise in $1,25(OH)_2$vitamin D concentrations that accompanied the onset of lactation occurred in the presence of high blood lead concentrations. The rat, however, is not as sensitive as the child to lead-induced changes in heme metabolism and therefore may also be less sensitive to lead-induced changes in vitamin D metabolites. Consequently, we conducted a study in rabbits, a species that is sensitive to lead-induced changes in the heme system.

In our study, which involved exposing rabbits to lead from 9 weeks of age (the day of weaning) to 26 weeks of age (when their body weights began to plateau), FEP concentrations increased fourfold and sixfold at blood lead concentrations of 45 and 55 μg/dL in rabbits receiving diets containing 200 and 400 ppm lead, respectively. In these same animals, lead exposure did not alter concentrations of vitamin D metabolites. With or without dietary lead exposure, concentrations of $1,25(OH)_2$vitamin D in the plasma of 10-week-old weanling rabbits ranged from 24-27 pg/mL. In 26-week old rabbits when growth had decreased, the concentrations of $1,25(OH)_2$ vitamin D were lower (12-15 pg/mL) and also unaffected by dietary lead exposure.

We conclude that the young growing rabbit did indeed show lead-induced changes in heme metabolism at blood lead concentrations similar to those in the children from the study of Rosen et al., but did not show corresponding lead-induced decreases in circulating $1,25(OH)_2$vitamin D concentrations. These results, along with those of our study in rats during pregnancy and lactation, suggest that an agent other than lead could have caused the decreases reported in the study of Rosen et al. If true, this finding is very important, since the U.S. Environmental Protection Agency is currently taking into consideration the results of Rosen et al. in setting a standard for lead exposure.

Influence of Pregnancy and Lactation on Cadmium Toxicity. We are currently examining the effects of cadmium, diet, and reproductive history on the etiology of Itai-Itai disease in Japanese women. This disease, characterized by extensive skeletal demineralization, is attributed in part to environmental exposure to cadmium in rice. The highest incidence of this disease has occurred in postmenopausal women with a history of multiple childbirths (average, six children). Our study examines for the first time the role of cadmium exposure during multiple pregnancies in generating toxic responses. Three groups of female mice (260-340 mice/group) were given diets containing 0.25, 5, or 50 ppm cadmium. At each cadmium level, mice received either a sufficient diet or an "Itai-Itai house-

hold" diet (deficient in certain vitamins, minerals, and fat). Half of the females were bred for six consecutive pregnancy/lactation (PL) cycles; the remaining females were nonpregnant (NP) controls. Cadmium intake and reproductive end points were monitored throughout the experiment. PL and NP females were sacrificed in all subgroups at the end of selected numbers of pregnancy/lactation cycles. Kidney, liver, femur, lumbar vertebrae, mammary tissue, duodenum, jejunum, and urine samples were taken for histological and/or biochemical evaluation. After six successive cycles, mice were oophorectomized to bring about hormonal changes characteristic of the postmenopausal period in humans.

Effects of cadmium exposure on reproduction in the dams, and on mineral content of bones of the dams and nonpregnant control mice, were reported last year. During this past year, we completed analysis of the bones of cadmium-exposed oophorectomized and sham-operated control mice. Our results show that loss of mineral in the axial skeleton was significantly enhanced by cadmium exposure in oophorectomized mice. At 50 ppm Cd, the lumbar vertebrae (L1-L5) of oophorectomized mice were 10% lower in dry weight and 20% lower in ash weight and calcium content than in sham-operated controls, and 9 to 10% lower in bone mineral density (i.e., ash/dry weight and calcium/dry weight ratios). These changes were greater in the lumbar vertebrae than in the femurs, and greater at 50 ppm Cd than at 0.25 or 5 ppm Cd.

Our earlier results showed that cadmium exposure resulted in decreases in bone mineral density that were greater in multiparous than in nulliparous mice. We have therefore shown a potential role of cadmium exposure both during multiple periods of pregnancy and lactation and during the postmenopausal period in the etiology of Itai-Itai disease.

Effects of Cadmium and Nutrient Deficiences in the Maternal Diet on Reproduction. On the basis of results from the larger cadmium experiment described above, we concluded that the diet consumed by the dam during a given pregnancy/lactation experience, rather than her past dietary exposure, may determine the effect of diet on reproductive variables. To test this hypothesis, we placed three groups of female mice on either (1) a sufficient diet low in cadmium (0.25+), (2) a deficient (Itai-Itai) diet low in cadmium (0.25-), or (3) a sufficient diet containing 50 ppm Cd (50+). We compared the reproductive parameters measured during a first pregnancy/lactation period for mice in the latter diet groups with those measured for the same mice during a second PL period when all three groups were given the control 0.25+ diet. The second PL period was initiated after 135 days of exposure to the above diets. Of the parameters measured, pup growth during lactation was affected most by dietary differences: at weaning, pups of dams on the 0.25- and 50+ diets weighed only 9 g, whereas the pups of dams on the control 0.25+ diet weighed 14 g. During the second PL period, however, pups of all dams weighed 13 to 14 g at weaning independent of past dietary exposure. Such results demonstrate the importance of proper maternal nutrition during a given pregnancy/

lactation experience and the lesser importance of prior exposures.

Studies with Plutonium and Other Actinides.

Gastrointestinal Absorption of Plutonium and Other Actinides. Our current aims are to (1) validate a dual-isotope method for determining the GI absorption of plutonium and uranium in the adult baboon that does not involve sacrificing the animal, (2) use this method to determine the GI absorption of plutonium and uranium under specific conditions of feeding and fasting, and (3) apply the method to measurements of GI absorption in the neonatal baboon. The "nonsacrifice" method requires simultaneous administration of two isotopes of a given actinide, one orally and one intravenously (iv) (DeGrazia et al., 1965, J. Lab. Clin. Med. 66, 822). GI absorption values are calculated from oral/iv isotopic activity ratios in separate samples of blood, urine, and/or biopsies of bone or liver. So far, two important results have emerged: (1) the dual-isotope method shows good promise as a method for determining GI absorption values of plutonium and uranium in the baboon, and (2) urine samples collected in the metabolism cage on Day 4 after administration are contaminated by feces to an extent that negates their use for calculation of GI absorption values of plutonium but not of uranium. The second result is explained by the fact that uranium is absorbed and excreted in urine to a much greater extent than plutonium, making fecal contamination a less likely problem for uranium.

With respect to the first result, the GI absorption value of plutonium in the first baboon obtained by direct analyses of tissues and excreta was 0.020% (0.018% retained + 0.002% excreted in urine). The mean value calculated from oral/iv isotopic ratios determined in separate samples of blood, urine, liver, and bone was 0.022%. Values calculated from individual ratios for 4-h blood, day 1 urine, liver, and caudal vertebrae were 0.022%, 0.027%, 0.022%, and 0.017%, respectively. Calculated values were thus consistent with one another and agreed with the one determined by direct analysis of the baboon at sacrifice. For uranium, the method appears to work equally well. For the first baboon, the GI absorption value obtained by direct analyses of tissues and excreta was 1.4% (0.2% retained + 1.2% excreted in urine). The mean value calculated by the dual isotope method was 1.2%. Values calculated from isotopic ratios in individual tissues were 0.8% (4 h blood), 1.1% (1-8 day cumulative urine), 1.5% (liver), and 1.4% (caudal vertebrae).

We have administered multiple isotopes of plutonium and uranium to three additional baboons and sacrificed two of them to complete validation of our method. Although tissues from the two sacrificed baboons are still being analyzed, analysis of blood, urine, and tissue biopsies is complete for all animals, allowing calculation of GI absorption values with the dual isotope method. As with the first baboon, results from the additional baboons indicate good agreement between values calculated from individual tissue samples. Mean GI absorption values calculated for all administrations of plutonium were 0.23 ± 0.15% for fasted adult baboons (0.02-0.60% range, five administrations) and 0.012 ± 0.011% for fed adult

baboons (0.004-0.02% range, three administrations). The GI absorption of uranium in fasted adult baboons was 4.5 ± 2.8% (1.3-5.9% range, four administrations). These GI absorption values for plutonium are nearly identical to those in fasted and fed adult mice, respectively.

Metabolic Behavior of Plutonium in the Dog: Comparison of Gastrointestinally Absorbed Plutonium with that of Intravenously Injected Plutonium. For the dual-isotope method to apply, the intravenously administered isotope must be metabolically equivalent to the absorbed portion of the orally administered isotope. Although this equivalence appears to hold true for the baboon, we decided to conduct an additional experiment in the dog, since in this species we can study the metabolic behavior of the two isotopes more closely by collecting multiple sequential blood and catheterized urine samples without requiring anesthesia. In this study, we simultaneously administered ^{239}Pu orally and ^{236}Pu intravenously to a fasted beagle (19-h fast). Results show that from day 0.2 to day 3, when more than 90% of each isotope was transferred from blood to tissues, the value of the $^{239}Pu/^{236}Pu$ activity ratio in blood did not change: values were 0.59, 0.55, 0.55, and 0.54 at 0.2, 1, 2, and 3 days after plutonium administration, respectively. In addition, the activity ratio in blood (mean, 0.56) was essentially the same as that in the 1, 2, and 6 day urine samples (0.57, 0.59, and 0.60, respectively) and in the liver (0.46) and bone (0.52) obtained at sacrifice of the dog. Furthermore, the GI absorption value calculated for this dog by the dual-isotope method was 0.08%, a value similar to the mean value we determined earlier in five fasted dogs (0.07%) (Toohey et al., 1983, Radiat. Res. 97, 373-379). These data show the metabolic equivalence of ingested and injected plutonium in the beagle and support the above results in the baboon.

Effect of Humic Acid Complexation on Plutonium GI Absorption. Since a number of drinking waters contain significant levels of humic acids, their effect on the GI absorption of plutonium needs to be considered in setting limits for exposure to plutonium in drinking water. We exposed groups of mice in the fed and fasted condition to solutions of plutonium complexed to humic acid (2×10^{-10} M ^{238}Pu, 15 ppm humic acid). According to preliminary results, the GI absorption of plutonium complexed to humic acid was 0.03% in fasted mice (n = 2) and was 0.005% in fed mice (n = 1). Humic acid complexation therefore appears to decrease the GI absorption of plutonium, because the above values are each about fivefold lower, respectively, than the values for fasted and fed mice given plutonium in 0.01 M $NaHCO_3$ medium.

Directions for the near future include (1) microradiographic analysis of bones from mice after ovariectomy; (2) study of metallothionein induction during pregnancy and lactation in mice as a possible explanation for identified changes in cadmium metabolism, and (3) completion of the validation of the dual-isotope method and use of the method to study the GI absorption of plutonium and uranium in neonatal and partially fasted adult baboons.

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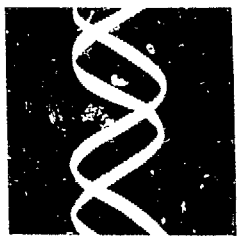
Hematological Responses to Arsine Exposure: Quantitation of Exposure Response in Mice
Fundam. Appl. Toxicol. 5, 499-505 (1985)

Accepted as of June 30, 1985

Bhattacharyya, M. H., D. A. Sellers, and D. P. Peterson
Postlactational Changes in Cadmium Retention in Mice Orally Exposed to Cadmium during Pregnancy and Lactation
Environ. Res.



21. NEUROBEHAVIORAL CHRONOBIOLOGY: ACTIONS OF HIGH-STRENGTH 60-Hz ELECTRIC FIELDS ON CIRCADIAN RHYTHMS

C. F. Ehret, Principal Investigator
K. R. Groh, and D. J. Fowler*

The objectives of this research are to (1) provide more information on how and why exposure of small rodents to high-strength 60-Hz electric fields produces circadian phase shifts and other alterations of the normal circadian waveform, and (2) determine how these effects that compromise the circadian integrity of the subject can be extrapolated across species. This effort is subdivided into three tasks: (1) to characterize the circadian rhythm effects at lower field strengths than those previously reported (135 kV/m) in the white-footed mouse Peromyscus leucopus; (2) to determine whether the previously reported absence of circadian effects in the rat was the result of the use of inappropriate exposure and entrainment conditions; and (3) to characterize the environmental and exposure conditions of defined circadian effects, including recovery and prevention.

For these studies, rats are housed singly in automated systems that allow strict control of inputs (food, light, toxicants) and continuous monitoring of physiological information over prolonged periods. This information produces circadian "signatures"--finely detailed waveforms of physiological oscillations of circadian and ultradian periods, which are derived from several end points, including gross motor activity and either core body temperature or respiration. After baseline "signatures" are obtained, the experimental protocol that includes electric-field exposure is introduced.

Electric Field Effects on Circadian Rhythms in Small Rodents.

In an earlier study, a comparison of the sensitivity of small rodents to arousal by 60-Hz electric fields revealed significant interspecies differences. The white-footed mouse Peromyscus leucopus was more sensitive than either the white rat (Charles River, SD) or the hairless mouse (HR/Temple, SKH). P. leucopus was therefore chosen to determine whether field exposures sufficient to produce arousal could also cause circadian phase shifts. Circadian oscillations in motor activity and in amounts of carbon dioxide produced were con-

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tinuously monitored before, during, and after a brief exposure (1 h or 9 h to 60-Hz electric fields at field strengths of 135 kV/m). Before exposure, the animals were entrained by light-dark cycling (12 h light, 12 h darkness). On the exposure day and for 5-6 days thereafter they were kept in darkness. Exposure for 1 h early in their active phase induced a significant phase delay, averaging 2.9 h, in circadian rhythms of the end points measured. Exposure during early inactive phase caused no phase shift but produced "splitting" (dissociation of the normally slightly bimodal active phase into two distinct peaks of activity, ultimately 180° or 12 h apart). Exposure for 9 h during the animal's active phase resulted in phase delays that averaged 4.6 h, and in occasional episodes of torpor. The degree of circadian phase shift was highly correlated with the magnitude of arousal in the animals exposed to the electric field for 9 h. At lower field strengths (100 kV/m), significant alterations in circadian waveforms including phase shifts, dyschronism, and torpor were seen in mice exposed during early or late subjective night. Mice exposed to fields during the middle of their active or inactive phase seldom showed any change in waveform. All responses of mice exposed to 100 kV/m were significantly lower than those exposed to 135 kV/m.

In this past year, we initiated a comparable series of experiments on the capacity of high-strength 60-Hz electric fields to violate the circadian integrity of the subject in another rodent species, the laboratory white rat. We modified our field-exposure and data acquisition facilities for the rat to enhance the precision and sensitivity of activity and temperature measurements. In addition, we have begun a series of experiments designed to answer the following questions: In the rat, do electric fields also cause phase shifts? What is the full shape of the generalized circadian phase response curve? Under what conditions can dyschronism be induced and/or prevented following arousal by 60-Hz electric fields? An analysis of cogent effects reported to date in the literature dealing with electric fields indicates that all known long-term health-risk actions of high-strength electric fields can be attributed to their action as circadian zeitgebers. These questions are all highly relevant to the evaluation of the health risk to humans of high-strength electric fields.

Publications

Accepted as of June 30, 1985

Cahill, A. L., and C. F. Ehret
Chronobiological Consequences of Various Shiftwork Schedules
Proceedings of the Fifteenth International Conference of the International Society for Chronobiology, Minneapolis, MN, September 13-18, 1981

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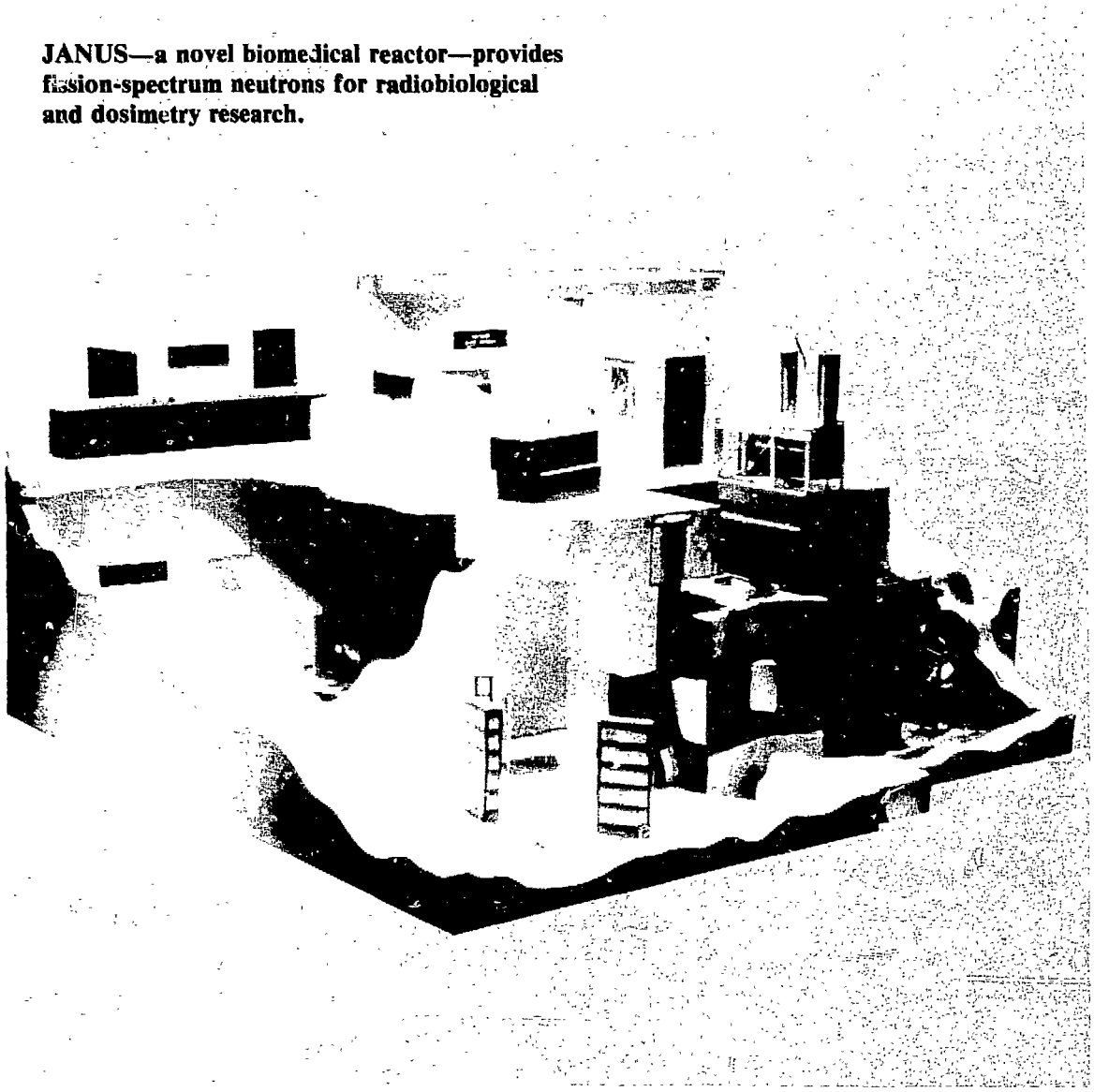
Circadian Manifestations of Barbiturate Habituation, Addiction, and Withdrawal in the Rat

Intl. J. Chronobiol.

Divisional Support Facilities, Services, and Activities



JANUS—a novel biomedical reactor—provides fission-spectrum neutrons for radiobiological and dosimetry research.

22. SUPPORT FACILITIES

Animal Facilities
Radiation Facilities
Human Radiobiology Counting Facilities
Computer Facilities
Interactive Computer Graphics System
Carcinogen Laboratory
Cytofluorograph Equipment
Two-Dimensional Electrophoresis Facilities
Chronobiology Facilities
Instrument Design and Maintenance Facility

The specialized facilities available in the Division of Biological and Medical Research provide outstanding and diverse capabilities for interdisciplinary research. Included are an accredited animal facility; a broad spectrum of radiation sources and facilities that include the Janus neutron reactor, five ^{60}Co-gamma-ray sources, several x-ray machines, and a unique collection of ultraviolet and visible radiation sources and equipment for photobiological research; specialized whole-body counting facilities for human radiobiology studies; computer equipment ranging from mainframe to microcomputers, with a user-oriented computer graphics system; a newly constructed carcinogen laboratory; a cytofluorograph laboratory; specialized laboratories for research in two-dimensional electrophoresis and chronobiology; and a fully equipped instrument design and maintenance center. A number of these facilities are available to outside users, either as a result of collaboration with Divisional staff members or on a user-pays basis.

Animal Facilities

The 3700 m^2 Animal Facility, a major resource of the Division, is accredited by the American Association for Accreditation of Laboratory Animal Care. During 1984 and 1985, approximately half of the staff members of the Division were engaged in studies that involved more than 18,000 rodents and other laboratory animal species.

The facilities consist of four major components: (1) dog kennels with a capacity for approximately 700 dogs; (2) two building wings consisting of 47 rooms for housing small laboratory animals; (3) service areas that 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, a histopathology laboratory for processing tissue specimens, a necropsy room, diagnostic x-ray rooms, and two specially equipped rooms for treating animals with radioactive materials, toxic chemicals, and carcinogens.

In addition to the resources listed above, four of the five Divisional ^{60}Co gamma-ray-exposure facilities, dedicated to whole-body irradiation studies in animals, are physically related to the animal facilities to provide for long-term, live-in exposures of animals.

To assure a supply of high-quality, healthy animals, the Animal Facilities maintain an active breeding program. Most of the rodents, particularly mice, and all dogs are bred in-house. The beagle colony, used primarily for low-level radiation studies, has been a closed colony since 1960. Extensive computerized records are maintained on all aspects of this colony, as well as on the rodent breeding program.

An expert staff, under the direction of a Doctor of Veterinary Medicine, manages, monitors, treats, and evaluates the animals and provides a complete range of services to users of the experimental animals. Trained Animal Care Specialists provide routine animal care.

Radiation Facilities

The Divisional radiation facilities include a number of gamma, neutron, x-ray, and ultraviolet radiation sources with accompanying areas for related equipment and for preparing, handling, and servicing the animals.

Five ^{60}Co irradiation facilities provide unique and versatile gamma-ray irradiation capabilities. These 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 0.001 to 10^4 R/min. Three of these gamma-ray facilities are committed to long-term irradiation of caged dogs, in 22 h daily exposures, at 0.6, 1.0, and 2.5 R/day, or at 5 to 40 R/day (adjustable). The remaining two facilities are used for irradiations of small animals and cell cultures. The low-level gamma-ray facility can be arranged, as required, to accommodate studies involving exposure rates from 1 mR/min to 30 mR/min. The versatile high-level gamma-ray facility can provide uniform exposures of 300 to 10,000 R/min to volumes of less than 25 cm^3. Any exposure rate between 20 mR/min to 30 R/min can be delivered to experimental material placed on isodose arcs.

The Janus research reactor, which is dedicated specifically to biological research, provides fission-spectrum neutrons with a mean energy of 0.85 MeV. Available dose rates range between 0.002 and 80 cGy/minute, with remarkably low contamination from gamma radiation (between 3 and 4% of the total absorbed dose). During 1984, the original fission converter assembly was replaced; dosimetric studies are currently in progress to compare neutron spectra, microdosimetry spectra, and neutron/gamma-ray dose rates at all locations in the irradiation room before and after the converter assembly replacement.

The Division maintains two x-ray therapy machines for experimental irradiations, a General Electric Maxitron (250 kVp) and a Norelco-Müller MG 300 (300 kVp).

The nonionizing radiation facility consists of a collection of sources of well-characterized, unusually high-intensity ultraviolet and visible radiations, extending from 254 nm through to the red end of the visible spectrum. Isolated band and high-pressure mercury arc lines throughout this range are available in extremely high energies with precisely defined doses. The sources are 2,500 and 6,000 W high-pressure xenon and mercury-xenon arc lamps, coupled to predispersion and collimating optics and high-intensity Bausch and Lomb and Jobin-Yvon monochromators. Accessories are available for the irradiation of cells under controlled conditions of temperature and gas phase.

Human Radiobiology Counting Facilities

The low-background counting center is designed to measure internal radioactivity in living subjects. Stationary detectors used for low-level *in vivo* gamma-ray spectroscopy and low-level photon measurement are located in three shielded rooms contained in a large underground "vault," and mobile counting equipment is contained in a trailer. Special shielding of the vault reduces the cosmic ray intensity to about half of its value outside at ground level. The 200-mm-thick steel walls of each room attenuate local gamma radiation to reduce the background response of the detectors and a 3 mm-thick lead covering of all internal surfaces absorbs scattered radiation and further reduces the background at low photon energies. Activated charcoal filtration of the vault air removes naturally occurring radon to reduce contributions to, and fluctuations in, the background counting rates from this source. An above-ground reception room, an elevator for transport between ground level and the anteroom of the vault, shower and dressing rooms, and an airlock complement the facility.

The first room contains two 292-mm- by 102-mm-thick NaI(Tl) detectors, one mounted above and one below a horizontal bed. These crystals are used to determine the longitudinal distribution of radioactivity in the body ("profile scans") and also to detect small amounts of gamma activity in localized areas such as the chest. The second room contains a 203-mm-long by 152-mm-diameter cylinder of NaI(Tl), and measurements are taken with the subject lying in an arc of radius 1.5 m, or sitting in a tilting chair. The third room is used to measure low-energy photon emitters, such as americium-241 and plutonium-239. Two detectors are used. The first is a 180-mm-diameter proportional counter filled with a mixture of xenon and methane, and the second is a "phoswich," consisting of a 3-mm-thick NaI(Tl) crystal backed by a 50-mm-thick CsI(Tl) crystal. Interactions in the two scintillators are distinguished by the rise times of their output pulses, and the CsI(Tl) crystal is used in an anticoincidence or Compton-suppression mode.

A separate smaller vault of similar construction is used to measure radioactivity in small- to medium-sized samples. Detectors include a low-background beta-particle counter, a low-background gamma-ray spectrometer, and a gamma-ray spectrometer with an automatic sample changer. The radiation shields of the gamma-ray spectrometers are supplied with radon-free air from the same source as the large vault.

Field measurements are taken with a whole-body counting system consisting of a shadow shield detector, multichannel analyzer, and electronic devices installed in a 7.6-m-long semitrailer. The radiation detector is a 204-mm-diameter by 102-mm-thick NaI(Tl) crystal, coupled to three photomultiplier tubes. For measurement, the patient reclines on a padded plate, which is moved underneath the detector by a chain drive mechanism, so that each measurement is an integrated scan.

Computer Facilities

Computer facilities for general use in the Division are built around the ANL central IBM-3033 computers plus a 3042 attached processor, 1.9×10^{10} bytes of online disk and drum storage, 26 magnetic tape drives, 55 Remote Job Entry (RJE) stations, and network connections to research centers in North American and Europe. These computers and associated systems are accessed from the Division interactively via eight dedicated terminals and numerous dial-up terminals. Batch access is principally via a dedicated RJE station with a 1000 cards per minute reader and an 1100 lines per minute printer.

The Divisional PDP-11/44 computer is also used as an additional RJE station and provides file transfer capability. This computer is being expanded to provide more than 750 megabytes of disk space and will service four dial-up ports and 36 dedicated terminal lines, 13 of which will be graphics terminals. Dedicated lines will also provide data paths from other data acquisition computer systems in the Division.

MINITAB and GLIM statistical packages and RDM and ORACLE data base management packages provide the main software capabilities. A Hewlett Packard eight-color pen plotter with continuous paper feed supplies batch graphics capability, from graphic files compatible with the IBM-3033 computers.

Interactive Computer Graphics Equipment

Interactive computer graphics capabilities are provided by an Evans and Sutherland Computer Corporation PS300 Picture System, which is a black-and-white, high-resolution, vector graphics terminal. The terminal contains a Magnavox 6800 microprocessing chip and 1 Mbyte of memory; it has a 5.25 in. floppy disk drive and can be programmed as a stand-alone computer with an internal language similar to PASCAL. This terminal is equipped with a rotating drum stereo attachment (Terabit) so the object appears in three dimensions and can be manipulated (translated, rotated, etc.) in three dimensions. Operating as a terminal emulator on a host computer like the VAX 11/780, the terminal can accept data passed at 19,200 baud from the host and display it in graphics mode as a set of line vectors; the PS300 can display 80,000 black-and-white vectors simultaneously. The FRODO molecular modeling program for displaying, building, and manipulating molecules, is now operational. It enables the display of electron density maps from protein crystals as contoured volumes and also

the display of chemical models as stick figures imposed upon the maps. Also available on the PS300 system is the provision to display protein structures for which coordinates are stored in the Brookhaven National Laboratory Protein Data Bank.

Carcinogen Laboratory

Our new Carcinogen Laboratory, completed in the summer of 1985, was designed to comply with current and projected federal, state, and local regulations on the handling of hazardous material. It contains 53 m^2 of isolated work space, subdivided into a storage vault and low-level and high-level workrooms for sample preparation. A decontamination room, shower room, and change room are also provided. The facility was designed to (1) control personnel traffic into, within, and out of the different areas; (2) maintain negative pressure gradients within each of the internal areas relative to other areas of the building and the external environment; (3) provide specialized equipment for the safe handling and containment of hazardous materials; and (4) provide work areas with impervious surfaces for ease in decontamination. A once-through ventilation system with activated charcoal and high-efficiency particulate air filters is designed to contain any contaminants that pass through the filters in the primary containment area or are accidentally released from the work areas within the facility. A telephone communication system between several work areas and between work areas and an outside observation area are provided so that strict operating procedures can be maintained without limiting interactions and efficiency.

Cytofluorograph Equipment

The Division maintains a flow cytometry system run by a trained operator. This Ortho Instruments dual laser cytofluograph with a "2150" computing system and argon ion and krypton ion lasers can be used for a wide variety of studies including high-resolution determinations of fluorescent DNA distributions in suitably stained cells, as well as light scatter properties and immunofluorescence with fluorescent-dye conjugated monoclonal antibodies on both fixed or viable cells. An attached cell sorter can be used to selectively collect a desired population of cells. All operations are computer controlled and data can be stored, displayed, printed, and analyzed with a microcomputer.

Two-Dimensional Electrophoresis Facilities

Prototype equipment for high-resolution two-dimensional protein mapping was developed in the Division. Our current system, the Argonne ISO-DALT electrophoretic system, can handle more than 100 two-dimensional analyses per day. Semiautomatic photography provides a record of all analyses and maps for publication. Two scanners are available to acquire and digitize images, which are then processed on a

dedicated VAX computer and displayed in high-resolution color. TYCHO programs have been developed for image background subtraction, photographic response correction, streak subtraction, and distortion compensation. Work stations allow experimental patterns to be intercompared with other stored experimental or reference patterns, and both qualitative and quantitative differences can be noted.

Chronobiology Facilities

The automated, computer-controlled data acquisition facilities used for research in chronobiology monitor and collect several types of data: body temperature, respiration (animal and cellular), animal activity, animal weight, and food consumption.

For monitoring of body temperature, a miniature temperature radio-telemeter is implanted intraperitoneally in each animal. The device transmits temperature data by a pulse-modulated system for collection by a two-plane antenna surrounding the animal cage.

For respiration monitoring, small animals or cell cultures are placed in sealed respiration chambers. Gas flow is monitored automatically by mass flow transducers and controlled by a computer-activated valving system. Atmospheric gases of known concentration are passed into the chambers and then sampled periodically for carbon dioxide and oxygen concentrations.

Activity and weight of individually caged animals are monitored through displacement of a force transducer. The generated signal is amplified and the ac and dc components separated. The dc signal variation is time-damped (animal weight), and the ac component is squared and integrated (animal activity).

Each animal can be provided with two different diets, each available from a food hopper suspended from a force transducer. At programmed times, the food hoppers are weighed and the amount of food remaining in each is recorded.

Instrument Design and Maintenance Facility

Design, construction, and testing of specialized equipment is provided for the Division. Trained electronics specialists operate and service high-performance liquid chromatography systems, gas chromatographs, automatic liquid scintillation spectrometers, automatic gamma spectrometers, visible and ultraviolet spectrophotometers, clinical blood analyzers, particle sizing and counting systems, teletype printers, cytofluorographs, data acquisition systems, and Janus reactor instrumentation.

Service is also provided to staff members in the selection and purchase of new instrumentation, as well as the modification of existing instruments to fulfill special requirements. If required instrumentation is not available commercially, new instrumentation is designed and, if appropriate, patented. Maintenance, servicing, and calibration of equipment is also provided.

23. EDUCATIONAL ACTIVITIES

Postdoctoral Training
Faculty Research Participations
Graduate Programs
Undergraduate Training

Postdoctoral Training

During 1984-1985, a total of 17 postdoctoral appointees and research associates contributed to the research programs of the Division.

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

Sudhir Bedarkar	Cornell University	E. M. Westbrook
James M. Burcham	Iowa State University	C. Peraino
Chong-Hwan Chang	University of Pittsburgh	M. Schiffer
Frank R. Collart	Medical College of Ohio, Toledo	E. Huberman
Michael L. Cunningham	University of Arizona	C. A. Reilly, Jr.
James P. Hardwick	Illinois State University	E. Huberman
Yoshimi Homma	University of Tokyo, Japan	E. Huberman
Atsushi Ito	University of Tokyo, Japan	M. J. Peak
Kaoru Kiguchi	Jikei University School of Medicine, Tokyo, Japan	E. Huberman
Nobuo Kubota	Yokohama City University School of Medicine, Japan	C. K. Hill
Ingrid R. Marshall	University of Karlsruhe, Karlsruhe, West Germany	D. Grahn
Vaman M. Naik	University of Michigan	M. Schiffer
Carol T. Oravec	Ohio State University	E. Huberman
Mark S. Swanson	University of Nebraska at the Medical Center	D. A. Haugen
Maria E. van Beek	State University, Utrecht, The Netherlands	D. Grahn
Robert L. Wells	Colorado State University	A. Han
Ti-Sheng Young	Duke University	E. M. Westbrook

Faculty Research Participations

Argonne and the Division have long involved faculty members on sabbatical leaves from colleges and universities and staff members from industrial laboratories in Argonne activities. During 1984-1985, four Visiting Scientists, 11 Faculty Research Program Participants, and three Faculty Research Leave at Argonne Participants conducted research in the Division. The two latter programs are administered through the Argonne Division of Educational Programs (DEP). All of these appointments enable faculty members to participate in the research of the Laboratory to broaden their perspectives; they also allow university and Argonne scientists to develop rapport and explore mutual interests. In addition, DEP has a program involving summer research participation by high school teachers; during this time, the Division had one such participant. The names of the Visiting Scientists, Faculty Research Participants, Faculty Research Leave at Argonne Participants, and the High School Faculty Research Participant, their schools, and their staff sponsors are as follows:

William E. Boernke*	Nebraska Wesleyan University	C. Peraino
Clinton H. Dixon‡	Ft. Valley State College, Georgia	H. E. Kubitschek
Dona J. Fowler‡¶	Western Michigan University	C. F. Ehret
Frederick A. Giere¶	Lake Forest College	N. G. Anderson
Malkiat S. Guram‡	Voorhees College	M. H. Bhattacharyya
Richard M. Hyslop‡	Illinois Benedictine College	C. S. Borso
Donald K. Jasper‡	Illinois Institute of Technology	T. E. Fritz
Paul G. Koutnik**	Wheaton-Warrenville Middle School	D. J. Grdina
Florence S. Lewis‡	Cheyney State College	E. Huberman
Sakan Maeda*	Kobe University of Medicine, Kobe, Japan	E. Huberman
Gerald A. Murison¶	Florida International University	E. Huberman
Biserka Nagy*	Central Institute for Tumors and Allied Diseases, Zagreb, Yugoslavia	D. J. Grdina
Frank H. Pascoe‡	College of St. Francis	N. G. Anderson
Robert M. Roth‡	Illinois Institute of Technology	M. J. Peak
Daniel Solaiman‡	Dubuesne University	M. H. Bhattacharyya
Juarine Stewart‡	Atlanta University	D. A. Haugen
Marvin Stodolsky*	Bosphorus University, Istanbul, Turkey	E. Huberman
Harold J. Van Kley‡	Trinity Christian College	C. Peraino

*Visiting Scientist.
‡Faculty Research Participant.
¶Scientist in Residence (Faculty Research Leave at Argonne).
**High School Faculty Research Participant.

Graduate Programs

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

Mary E. Shackelford	University of Illinois, Chicago	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 1984-1985, two students held this appointment in the Division:

Greg S. Spicer*	Texas Technical University	N. G. Anderson
Jeffrey J. Wheat	George Williams College	R. E. Toohey

In addition, one graduate student carried out research in the Division as Resident Student Associate, supported directly by the Division:

Marian F. Papiernik	St. Xavier College	C. F. Ehret

Undergraduate Training

During 1984-1985, a total of 59 college undergraduates received training in the Division of Biological and Medical Research through the Spring, Summer, and Fall Undergraduate Research Participation Programs or the College Co-op Program, sponsored by the Division of Educational Programs. The students, their schools, and their staff supervisors are listed below:

Spring Program 1984

Frederick Domann	University of Wisconsin, Platteville	T. M. Seed
Silvia Pastor	Saginaw Valley State College, Michigan	C. K. Hill
Lorna A. Pehl	Seattle Pacific University	F. R. Kirchner
Paul J. Quiram	St. John's University, Minnesota	C. F. Ehret
Maxime Viard	Chicago State University	Y. E. Rahman
David E. Werkmeister	Westmar College, Iowa	E. Huberman

*In October 1984, he began as a Laboratory Graduate Participant.

Pamela A. Wexler	Beloit College, Wisconsin	F. R. Kirchner
Thomas L. Winder	University of Northern Iowa, Cedar Falls	C. F. Ehret

Summer Program 1984

Michael K. Albertson	University of Illinois, Champaign	N. G. Anderson
Gregory S. Culen	St. Xavier College, Chicago	F. R. Kirchner
Dwana Davis	Benedict College, Columbia, South Carolina	H. K. Kubitschek
Antonio Devia	University of Texas, Arlington	M. J. Peak
Steven Diebold	University of Chicago	F. R. Kirchner
Frederick Domann*	University of Wisconsin, Platteville	T. M. Seed
Edward J. Dunphy	University of Chicago	M. Stodolsky
Danielia Falcon	Parkland Jr. College, Champaign, Illinois	T. E. Fritz
Jeffrey Goldman	Northwestern University	M. Schiffer
Brian E. Helmer	Western Michigan University, Kalamazoo	C. F. Ehret
John Holland	Carroll College, Helena, Montana	C. K. Hill
Jennifer S. Johnson	Illinois Institute of Technology	M. J. Peak
Steven Kliewer	Brown University, Providence, Rhode Island	M. Schiffer
Patricia Small	Tougaloo College, Michigan	Y. E. Rahman
Thomas Winder*	University of Northern Iowa, Cedar Falls	C. F. Ehret

Fall Program 1984

Heidi Dare	Albion College, Albion, Michigan	M. H. Bhattacharyya
Pamela Donovan	College of St. Thomas, St. Paul, Minnesota	T. M. Seed
Susan M. Giovanazzi	St. Xavier College, Chicago	M. J. Peak
Mark C. Harbeck	Illinois Benedictine College, Lisle	T. M. Seed
Thomas Hickock	Macalester College, St. Paul, Minnesota	C. A. Reilly
Michael Ochocki	Northern Illinois University	C. F. Ehret
James Russell	Morris Brown College, Atlanta	H. E. Kubitschek
Thomas Winder*‡	University of Northern Iowa, Cedar Falls	C. F. Ehret

*Also participant in the Spring 1984 Undergraduate Research Participation Program.
‡Also participant in the Summer 1984 Undergraduate Research Participation Program.

Spring Program 1985

David M. Babasick	University of Bridgeport	C. K. Hill
Pamela K. Donovan*	College of St. Thomas, Minnesota	T. M. Seed
Barbara Einhorn	CWR University, Cleveland, Ohio	C. A. Jones
Sue Giovanazzi*	St. Xavier College, Chicago	M. J. Peak
Gregory Grose	Carroll College, Helena, Montana	C. F. Ehret
Mark C. Harbeck*	Illinois Benedictine College, Lisle	T. M. Seed
Peggy Janiszewski	St. Francis College, Joliet	C. A. Reilly
John B. Nicholas	Illinois Benedictine College, Lisle	C. F. Ehret
Anthony Pietroniro	Barry University, Miami Shores	M. H. Bhattacharyya
Lawrence D. Powell	Chicago State University	Y. E. Rahman
Chrysanthy M. Tsifutis	Grinnell College	H. E. Kubitschek
Lisa A. Urda	Indiana University of Pennsylvania	R. E. Toohey
Terry Uwagboi	Chicago State University	H. E. Kubitschek

Summer Program 1985

Christine Blaumueller	Northwestern University	C. A. Reilly
Steven Diebold‡	University of Chicago	C. A. Reilly
Barbara Einhorn¶	CWR University, Cleveland	C. A. Jones
Steven E. Gay	Yale University, New Haven	E. Huberman
Mark C. Harbeck*¶	Illinois Benedictine College	T. M. Seed
Susan Jacob	University of Chicago	C. A. Reilly
Jennifer Johnson‡	Illinois Institute of Technology	M. J. Peak
David Kardatzke	St. Xavier College, Chicago	J. H. Stebbings
Joseph S. Koboszka	Loyola University	C. K. Hill
Dale S. Lachman	Northern Illinois University	C. F. Ehret
Timothy Lowry	Hastings College	R. E. Toohey
Joseph L. Melendres	University of Chicago	D. V. Tolle
Christina Mihaly	Bowling Green State University	S. H. Barr
Henry Pak	University of Illinois	C. F. Ehret
Melissa Peterson	Parkland College	T. E. Fritz
John B. Nicholas	Illinois Benedictine College	C. F. Ehret
Lawrence D. Powell¶	Chicago State University	Y. E. Rahman
Miguel A. Schmitz	Carroll College	H. E. Kubitschek
Stephen Treacy	University of Notre Dame	D. J. Grdina
Patricia L. Winslow	Carroll College	H. E. Kubitschek

*Also participant in the Fall 1984 Undergraduate Research Participation Programs.
‡Also participant in the Summer 1984 Undergraduate Research Participation Program.
¶Also participant in the Spring 1985 Undergraduate Research Participation Program.

College Co-op Program

Rachael Casey	Westminster College	D. Grahn
Mary Lyn Deacon	Lewis University	E. M. Westbrook
Michael Olenek	Illinois Benedictine College	E. M. Westbrook
Cheri Pott	Trinity Christian College	D. V. Tolle
Bruce Ten-Haken	Trinity Christian College	C. Peraino
Larry Verhage	Trinity Christian College	E. M. Westbrook

24. DIVISIONAL SEMINARS

Visiting Speakers
In-House Speakers

The Division of Biological and Medical Research Seminar Committee for 1984-1985 was composed of M. H. Bhattacharyya (chairman), C. A. Jones, R. A. Schlenker, T. M. Seed, and E. M. Westbrook. T. M. Seed served as host for the in-house speakers. The 1985-1986 Committee is chaired by E. M. Westbrook and consists of M. H. Bhattacharyya, C. S. Giometti, D. J. Grdina, and R. A. Schlenker.

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

Visiting Speakers

Dr. Jun Minowada, Hines Veterans Hospital, Hines, IL
"Immunological Study of Human Leukemia-Lymphoma Cell Lines: Clinical and Theoretical Significances"
January 12, 1984

Dr. Lelia Diamond, Wistar Institute of Anatomy and Biology, Philadelphia, PA
"Structure Activity Relationship of Polycyclic Aromatic Hydrocarbons"
January 19, 1984

Dr. Janet Rowley, Department of Medicine, University of Chicago, Chicago, IL
"The Biologic Implications of Consistent Chromosome Abnormalities in Leukemia and Lymphoma"
January 24, 1984

Dr. Henry C. Pitot, McArdle Laboratory for Cancer Research, Department of Oncology, University of Wisconsin, Madison, WI
"Some Characteristics of the Stages in Hepatocarcinogenesis"
February 2, 1984

Dr. Alan P. Poland, McArdle Laboratory for Cancer Research, Department of Viral Oncology, University of Wisconsin, Madison, WI

"Mechanism of Biological Action of Division: The Dual Role of the Ah Locus Receptor"

February 9, 1984

Dr. Arend Kootstra, Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland

"The Importance of Chromatin Modifications in Neoplastic Transformation"

February 10, 1984

Dr. Frank F. Richards, Yale Medical School, Yale-New Haven Medical Center, New Haven, CT

"A Structural Basis for Antigenic Variation in African Trypanosomes"

February 23, 1984

Dr. Philip Mirkes, Department of Pediatrics, University of Washington, Seattle, WA

"Effects of Cyclophosphamide on Rat Embryo Development: An in vitro Analysis of Molecular Mechanisms of Teratogenesis"

March 1, 1984

Dr. Richard J. Albertini, Department of Medicine, University of Vermont, Burlington, VT

"Human Biomonitoring: The Use of T-Lymphocytes as Indicators of Somatic Cell Mutation"

March 8, 1984

Dr. Antonito T. Panganiban, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI

"Integration of Retrovirus DNA"

March 15, 1984

Dr. I. B. Kingston, MRC Laboratory of Molecular Biology, Cambridge, England

"Isolation and Sequence of Genes for Bovine Pancreatic Trypsin Inhibitor Using Synthetic DNA Probe"

March 22, 1984

Dr. Ronald B. Herberman, Department of Health and Human Services, Biological Development Branch, Frederick, MD

"Characteristics and Functions of Natural Killer Cells"

March 29, 1984

Dr. Ananda Chakrabarty, Department of Microbiology and Immunology, University of Illinois, Chicago, IL

"Microbial Technology for Enhanced Degradation of Environmental Pollutants"

April 5, 1984

Dr. Terry D. Allen, Department of Ultrastructure, Paterson Laboratories, Manchester, England
"Cellular Interactions in Long Term"
April 13, 1984

Dr. Frank J. Gonzalez, Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD
"Structure and Regulation of Two Mouse 3-Methylcholanthrene-induced Cytochrome P450s"
April 26, 1984

Dr. H. Yamasaki, International Agency for Research on Cancer, Lyon, France
"Intercellular Communication, Cell Differentiation, and Tumor Promotion"
May 7, 1984

Dr. Joseph Feder, Monsanto Company, St. Louis, MO
"The Development of Mammalian Cell Profusion Culture Systems"
May 24, 1984 (Joint BIM/EES Seminar)

Dr. L. Scott Cram, National Flow Cytometry Resource, Los Alamos National Laboratory, Los Alamos, NM
"New Developments in Flow Cytogenetics and Karyotype Instability in Tumorigenesis"
June 21, 1984

Dr. Jacques Laval, Institut Gustave Roussy (C.N.R.S.), Villejuif, France
"Repair of Alkylated DNA: Molecular and Biological Implications"
June 22, 1984

Dr. Tatsuo Suda, Department of Biochemistry, Showa University Dental School, Shinagawa-ku, Japan
"The Differentiating Action of Vitamin D Compounds in Hematopoietic Cells"
July 11, 1984

Professor Donald M. Engelman, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT
"Bacteriorhodopsin Folding: Theory, Biochemistry, and Neutrons"
July 27, 1984

*Dr. Hylton Smith, Biology Department, National Radiological Protection Board, Oxfordshire, England
"Application of Biological Research at NRPB to Radiological Problems"
September 27, 1984

*Informal seminar.

Dr. Mervyn Bibb, John Innes Institute, Department of Genetics, Norwich, England
"Gene Expression in Antibiotic Producing and Differentiating Streptomyces"
September 28, 1984

Dr. Michael G. Rossman, Department of Biological Sciences, Purdue University, West Lafayette, IN
"Structure, Function, and Evolution of Small Spherical RNA Viruses"
October 4, 1984

Dr. M. George Cherian, Departments of Pathology and Medicine, Health Science Center, University of Western Ontario, London, Ontario, Canada
"Metallothionein during Mammalian Development"
October 11, 1984

*Professor Detlef Gabel, Department of Chemistry, University of Bremen, Bremen, Federal Republic of Germany
"Neutron Capture Therapy of Tumors: Biological Basis and Development of Chemical Techniques"
October 18, 1984

*Dr. Frank T. Robb, Microbiology Department, University of Cape Town, South Africa
"Sanger Dideoxy DNA Sequencing Techniques Applied to Repetitive DNA of Very High G-C Content"
October 26, 1984

Dr. Stanley Prusiner, Department of Neurology, University of California, School of Medicine, San Francisco, CA
"Prions, Scrapie, and Dementia"
November 6, 1984

Dr. William E. Loewe, Lawrence Livermore Laboratory, Livermore, CA
"Revision of the A-bomb Survivor Dosimetry - Origin, Status, Expectations"
November 15, 1984

Professor Philip Low, Department of Chemistry, Purdue University, West Lafayette, IN
"The Structures and Functions of Band 3 of the Red Blood Cell and Other Plasma Membranes"
December 6, 1984

Dr. Stan D. Vesselinovitch, Departments of Radiology and Pathology, University of Chicago, Chicago, IL
"Kinetics of Mouse Hepatocarcinogenesis"
December 20, 1984

*Informal seminar.

Dr. Michael R. Thompson, University of Cincinnati, Department of Internal Medicine, Division of Digestive Diseases, Cincinnati, OH

"Heat-Stable Enterotoxins Produced by Toxigenic Escherichia coli"

January 10, 1985

Dr. Luka Milas, Department of Experimental Radiotherapy, M. D. Anderson Hospital and Tumor Institute, University of Texas System Cancer Center, Houston, TX

"Radioprotectors in Radiobiology and Radiotherapy"

January 15, 1985

Dr. G. Barry Pierce, Department of Pathology, University of Colorado Medical Center, Denver, CO

"Embryo In Regulation of Carcinoma"

January 24, 1985

Dr. Michael I. Lerman, Laboratory of Experimental Pathology, National Cancer Institute-FCRF, Frederick, MD

"Molecular Cloning and Characterization of Genes that Specify Sensitivity to Promotion of Neoplastic Transformation"

January 28, 1985

Dr. Dante G. Scarpelli, Department of Pathology, Northwestern University Medical School, Chicago, IL

"Rodent Pancreas - A Model for Transdifferentiation"

February 21, 1985

Dr. Harry V. Gelboin, Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD

"Monoclonal Antibodies for Analysis and Phenotyping of Cytochrome P-450"

February 28, 1985

Dr. J. Bernard Weinstein, Institute of Cancer Research, Columbia University, College of Physicians and Surgeons, New York, NY

"Molecular Mechanisms of Multistage Carcinogenesis"

March 7, 1985

Dr. Norman I. Krinsky, Department of Biochemistry and Pharmacology, Tufts University, School of Medicine, Boston, MA

"The Biology of Excited Oxygen Species"

March 14, 1985

Dr. J. Carl Barrett, National Institute of Environmental Health Sciences, Research Triangle Park, NC

"Possible Genetic and Cellular Targets for Chemically Induced Neoplastic Transformation"

March 21, 1985

Dr. Ruth Sager, Division of Cancer Genetics, Dana-Farber Cancer Institute, Boston, MA

"Anti-Oncogenes: The Next Frontier"

March 28, 1985

Dr. Richard Van Frank, Eli Lilly & Co., Inc., Indianapolis, IN

"Genetic Engineering and Contemporary Protein Chemistry Techniques for Analysis of Gene Products"

April 4, 1985

Professor Richard G. W. Anderson, Department of Cellular Biology, University of Texas Health Science Center, Dallas, TX

"Membrane Proteins Involved in Cellular Cholesterol Metabolism"

April 11, 1985

Dr. George Michalopoulos, Department of Pathology, Duke Comprehensive Cancer Center, Durham, NC

"Liver Regeneration Studies with Hepatocyte Cultures"

May 2, 1985

Dr. Hector F. DeLuca, Department of Biochemistry, University of Wisconsin, Madison, WI

"Molecular Mechanisms of Action of 1,25-Dihydroxyvitamin D_3"

May 9, 1985

Dr. Bernhard Ryffel, Pharmaceutical Division, Sandoz Ltd., Basel, Switzerland

"Present Knowledge on the Mechanism of Cyclosporine"

May 29, 1985

Dr. Graham Walker, Department of Biological Science, Stanford University, Stanford, CA

"Mutagenesis and Cellular Responses to DNA Damage"

June 18, 1985

Dr. Marvin Frazier, Pacific Northwest Laboratory, Richland, WA

"Role of Oncogenes in Radiation Carcinogenesis"

June 27, 1985

*Dr. John D. Harrison, National Radiological Protection Board, London, England

"Gastrointestinal Absorption of Actinides"

June 28, 1985

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*Informal seminar.

In-House Speakers

Dr. Eliezer Huberman
"The Induction of Cell Differentiation in Human Leukemia Cells by Specific Chemical Stimuli"
January 5, 1984

Dr. Thomas E. Fritz
"Late Effects of Protracted Whole-Body Irradiation"
February 16, 1984

Dr. Carl Peraino
"Studies of Multistage Hepatocarcinogenesis"
May 17, 1984

Dr. Y. E. Rahman
"Biotechnology of Targeted Drug Delivery"
May 31, 1984

Dr. Christopher A. Reilly
"Coal Conversion Health Effects"
September 20, 1984

Dr. Thomas M. Seed
"Cellular Bases of Divergent Pathological Responses under Protracted Radiation Exposure"
November 27, 1984

Mr. David V. Tolle
"Smears, Scrapings, and Squashes: A Clinical Study in Diagnostic Cytology"
January 3, 1985

Dr. Richard E. Toohey
"Radon Exposures in the Domestic Environment
February 15, 1985

Dr. David J. Grdina
"Effects of Radioprotectors on Cell Killing, Mutagenesis, and Transformation"
April 18, 1985

Dr. James M. Burcham
"Circadian Cycle in mRNA Levels in Rat Liver"
April 25, 1985

Dr. Michael L. Cunningham
"Biological and Photochemical Aspects of Superoxide Anion"
May 16, 1985

25. OUTSIDE TALKS BY DIVISIONAL STAFF*

Dr. Norman G. Anderson

High-Resolution Two-Dimensional Electrophoresis
International Conference on New Developments in Clinical Laboratory Instrumentation, Robert S. First, Inc., Boston, MA
January 16-17, 1984

New Techniques in Protein Chemistry
Twelfth International Congress of Clinical Chemistry, Rio de Janeiro, Brazil
April 30-May 4, 1984

Biotechnology: The Broader View
Chemistry Colloquium Series, Louisiana State University, Baton Rouge, LA
November 2, 1984

Computers and the Human Protein Index
The Computer Museum, Boston, MA
April 11, 1985

Dr. N. Leigh Anderson

Problems Associated with the Human Protein Index
Toward a Human Protein Map, CODATA Secretariat Meeting, Jerusalem, Israel
June 24-27, 1984

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

Review of High Resolution Protein Analysis
Third International Symposium on Two-Dimensional Electrophoresis Protein Mapping, Argonne National Laboratory, Argonne, IL
June 10-14, 1984

Identification and Classification of Cell Types Using Numerical Taxonomic Methods and 2-D Gel Data
Third International Symposium on Two-Dimensional Electrophoresis Protein Mapping, Argonne National Laboratory, Argonne, IL
June 10-14, 1984

Computers and the Human Protein Index
The Computer Museum, Boston, MA
April 11, 1985

Dr. Maryka H. Bhattacharyya

Effects of Cadmium and/or Diet on Reproductive Parameters in Female Mice Bred through Six Consecutive Cycles

Effects of Cadmium, Diet, and Reproductive History on Bone Parameters in Mice

Effects of Lead Treatment during Pregnancy and Lactation on Vitamin D Metabolism
Society of Toxicology Meeting, Atlanta, GA
March 12-16, 1984

Cadmium Transfer to Mouse Pups during Lactation: Role of Cadmium in Maternal Diet
Sixty-eighth Annual Meeting of the Federation of American Societies for Experimental Biology, St. Louis, MO
April 1-6, 1984

Cadmium and Itai-Itai Disease
Biological Science Department, Illinois Institute of Technology, Chicago, IL
March 5, 1985

Plutonium GI Absorption: Influence of Chemical Form and Animal Species
Speciation-85 Seminar, Speciation of Fission and Activation Products in the Environment, Oxford, England
April 16-19, 1985

A Dual-Isotope Method for Determining the Gastrointestinal Absorption of Plutonium and Uranium in Adult Baboons
Annual Meeting of the Health Physics Society, Chicago, IL
May 26-31, 1985

GI Absorption of Actinides
DOE Workshop on Actinide GI Absorption, Seattle, WA
June 24-26, 1985

Dr. Bruce A. Carnes

Habitat Utilization in a Prairie Rodent Community
Illinois State University, Department of Biological Sciences Seminar, Normal, IL
March 29, 1984

Dr. Michael L. Cunningham

Comparison of In Vivo and In Vitro Tests for Carcinogenicity of Complex Mixtures
Society of Toxicology Meeting, Atlanta, GA
March 12-16, 1984

Dr. Charles F. Ehret

The Induction of Circadian Phase Shifts and Dyschronism by Chronobiotically Active Drugs and Hormones
Ninth Annual Clinical Pharmacy Symposium, Tallahassee, FL
February 23-26, 1984

Circadian Clocks at the Base of Life
Keynote Address, Illinois Junior Science and Humanities Symposium, Southern Illinois University, Carbondale, IL
April 5, 1984

Consequences and Management of Circadian Phase Shift
Departmental Seminar in Biological Sciences, Marquette University, Milwaukee, WI
April 13, 1984

Ecology, Economics and Ecophilia: Bioethical Futures for Knowing, Managing, and Loving our Milieu
Chicago Advanced Seminar in Religion and Science, University of Chicago Meadville-Lombard Theological School, Chicago, IL
April 24, 1984

Better Industrial Production through Better Shift-Work ROTAS and Circadian Chronohygiene
American Industrial Hygienic Association Conference, Detroit, MI
May 22-25, 1984

Recent Advances in Chronobiology and Chronobiotechnology
Summer Institute for Teachers, Fermilab, Batavia, IL
July 5, 1984

Introduction to Circadian Rhythms in the Central Nervous System
Satellite Symposium on Circadian Rhythms, Ninth International Congress of Pharmacology, University of Bath, Bath, England
August 4, 1984

Actions of High-Strength 60 Hz Electric Fields on Circadian Rhythms in Small Rodents
International Workshop on Biological Effects of Electric Fields, Corsendonk Abbey, Corsendonk, Belgium
August 21, 1984

Circadian Clocks at the Base of Life: Recent Advances in Chronobiology and Chronobiotechnology
Fall Seminar Series, Fermilab, Batavia, IL
October 10, 1984

Workshop on Circadian Rhythms and Electric Fields
DOE Contractor's Review, St. Louis, MO
November 9, 1984

Dependence of Nuclear Reactor Safety on Sound Shift-work Practices
American Nuclear Society/European Nuclear Society, International Conference, Washington, DC
November 11-16, 1984

Circadian Clocks at the Base of Life: Recent Advances in Chronobiology and Chronobiotechnology
Biology Department, Illinois Institute of Technology, Chicago, IL
January 28, 1985

Introduction to Chronobiology and Chronobiotechnology
University of Dayton, Chautauqua Field Center, Dayton, OH
April 10, 1985

Chronobiotechnology and Chronobiological Engineering: Data Acquisition Methods in Annual Research
NATO Advanced Research Workshop in Chronobiotechnology and Chronobiological Engineering, Cardiff, Wales
April 22, 1985

Recent Advances in Chronobiology and Chronobiotechnology: Applications for Shift Workers and for World Travelers
CERN Colloquium, European Organization of Nuclear Research, Geneva Switzerland
April 30, 1985

Dr. Thomas E. Fritz

Late Effects of Protracted Whole Body Irradiation
University of Iowa, Department of Radiology Seminar, Radiation Research Laboratory, Iowa City, IA
February 28, 1984

Dr. Carol S. Giometti

Activation-Related Alterations in the Cytoskeletal Proteins of Human Platelets As Monitored by Two-Dimensional Electrophoresis
Sixty-eighth Annual Meeting of the Federation of American Societies for Experimental Biology, St. Louis, MO
April 1-6, 1984

Protein Changes in Activated Human Platelets

Tropomyosin Heterogeneity in Human Cells
Third International Symposium on Two-Dimensional Electrophoresis Protein Mapping, Argonne National Laboratory, Argonne, IL
June 10-14, 1984

Tropomyosin Heterogeneity in Human Skeletal Muscle
One Hundred and Ninth Annual Meeting of the American Neurological Association, Baltimore, MD
October 7-10, 1984

Dominant Lethal Mutation Rates Induced in Male Mice Exposed to Long-Term Once Weekly Neutron and Gamma-Ray Irradiation
Annual Meeting of the Environmental Mutagen Society, Las Vegas, NV
February 26-March 1, 1985

Dr. David J. Grdina

A Study of the Effects of WR-2721 and WR-1065 on Mutagenesis and Transformation
Thirty-second Annual Meeting of the Radiation Research Society, Orlando, FL
March 25-29, 1984

The Use of Automated Cell Sorting and Cell Separation Techniques in Radiation Biology
Rush Presbyterian-St. Luke's Hospital and Medical Center, Department of Radiology, Chicago, IL
April 8, 1985

Dr. David A. Haugen

Use of Mammalian Cells to Investigate Toxicologic Interactions for Responses to Complex Mixtures
Environmental Protection Association Symposium, Chapel Hill, NC
March 27-29, 1984

Isolation and Metabolic Activation of Mutagenic Aromatic Amines
Atlanta University, Biology Department, Atlanta, GA
March 1, 1985

Toxicologic Interaction of Neutral Components of a Coal Gasification Tar as Measured by Their Mouse Dermal Tumor-Initiating Activity

Comparative Cytotoxicity of Polycyclic Aromatic Hydrocarbons in Chinese Hamster Ovary Cells: Mechanism of Protection Against Cytotoxicity
DOE/OHER Complex Mixtures Program Contractors' Meeting, Burr Ridge, IL
June 17-18, 1985

Dr. Colin K. Hill

Qualitative and Quantitative Differences in Neoplastic Transformation Induced by Low Doses of ^{60}Co-Gamma Rays and Fission Spectrum Neutrons

Early and Late Events in Radiation-Induced Neoplastic Transformation in vitro
Thirty-second Annual Meeting of the Radiation Research Society, Orlando, FL
March 25-29, 1984

Role of Repair Processes in Neoplastic Transformation Induced by Ionizing Radiation in C3H 10T1/2 Cells
Conference, Carcinogenesis, a Comprehensive Survey: Cell Transformation Assays, National Institute of Environmental Health Sciences, Research Triangle Park, NC
September 11-15, 1984

Some Aspects of the Chemical Modulation of Neoplastic Transformation Induced by Ionizing Radiations in C3H 10T1/2 Cells
Pathology Department, University of Southern California School of Medicine, Los Angeles, CA
January 19, 1985

Amplification by TPA of the Low Level of Transformation Induced by ^{60}Co γ-Rays at 10 rad/day
Thirty-third Annual Meeting of the Radiation Research Society, Los Angeles, CA
May 6, 1985

Chemical Modulation of Neoplastic Transformation by High and Low LET Radiations
Norris Cancer Centre, University of Southern California School of Medicine, Los Angeles, CA
May 7, 1985

Dr. Eliezer Huberman

Induction of Mutations by Chemical Carcinogens at the Hypoxanthine Guanine Phosphoribosyl Transferase Locus in Human Epithelial Teratoma Cells
National Cancer Institute Board of Scientific Counselors; Bethesda, MD
March 1-2, 1984

Specific Nuclear Antigens Expressed in Differentiating Human Myeloid Leukemia Cells

Transformation Studies with Cryopreserved Syrian Hamster Embryo (SHE) Cells: An Interlaboratory Comparison
Seventy-fifth Annual Meeting of the American Association for Cancer Research, Toronto, Ontario, Canada
May 9-12, 1984

The Control of Mutagenesis and Differentiation in Cultured Human Cells by Chemicals that Initiate or Promote Tumor Formation
Conference, Assessment of Risk from Low Level Exposure to Radiation and Chemicals: A Critical Overview, Brookhaven National Laboratory, Long Island, NY
May 20-23, 1984

The Control of Cell Differentiation by Tumor Promoters in Cultured Human Cells
Institut Gustave Roussy Seminar, Villejuif, France
May 29, 1984

Control of Cell Differentiation in Leukemia Cells by Chemicals which Promote Tumor Formation
Ninth Annual Meeting of the Association of Cytogenetic Technologists, Chicago, IL
June 16, 1984

The Control of Cell Differentiation and Mutagenesis in Cultured Human Cells by Chemicals which Initiate or Promote Tumor Formation

Cell/Organ Specificity in Chemical Carcinogenesis
Tel Aviv University Seminar, Tel Aviv, Israel
November 1, 1984

The Control of Differentiation in Cultured Human Cells by Chemicals that Promote Malignant Cell Transformation
University of Texas System Cancer Center, Smithville, TX
January 22, 1985

Control of Cell Differentiation in Cultured Human Leukemia Cells by Chemicals that Promote Tumor Formation
University of Colorado Health Sciences Center, School of Medicine, Department of Pathology, Denver, CO
March 25, 1985

The Control of Differentiation in Cultured Human Leukemia Cells by Chemicals that Promote Cell Transformation
University of Wisconsin, Wisconsin Clinical Cancer Center, Department of Human Oncology, Radiobiology Section, Madison, WI
April 17, 1985

Cell Specific Activation of Chemical Carcinogens into Mutagens for Cultured Human and Rodent Cells
Fourth International Conference on Environmental Mutagens, Stockholm, Sweden
June 24-28, 1985

Dr. Carol A. Jones

The Frequency of Morphologically Transformed Colonies Induced by Chemical Carcinogens is Enhanced by 1α,25-Dihydroxycholecalciferol, the Biologically Active Metabolite of Vitamin D_3
Seventy-fifth Annual Meeting of the American Association for Cancer Research, Toronto, Ontario, Canada
May 9-12, 1984

Dr. Albert T. Keane

Radiation Damage Affects the Rate of Elimination of Radium by Man
Annual Meeting of the Health Physics Society, Chicago, IL
May 26-31, 1985

Dr. Herbert E. Kubitschek

Constancy of Buoyant Density of E. coli and Cultured Murine Cells

Variation in Buoyant Density during the Cell Cycle of Saccharomyces
Eighty-fourth Annual Meeting of the American Society of Microbiology, St. Louis, MO
March 4-9, 1984

Cell Growth in E. coli during Shifts to Richer Media

Buoyant Density Constancy of Cells of a Fission Yeast
Federation of European Microbiological Societies Symposium, Bacterial Morphogenesis 1984, Marseille, France
September 5-7, 1984

Cell Growth in E. coli during Shifts to Richer Media

Constancy and Osmoregulatory Control of Cell Buoyant Density
European Molecular Biology Association Symposium, Regulation of the Cell Cycle in Prokaryotes, Sede Boqer, Israel
September 9-13, 1984

Cell Growth and Buoyant Density Regulation
Indiana University School of Medicine, Gary, IN
January 29, 1985

Dr. Ingrid R. Marshall

Radiation Dose as Experienced by the Cell Nucleus
Thirty-third Annual Meeting of the Radiation Research Society, Los Angeles, CA
May 5-9, 1985

Microdosimetric Spectra Measurements of Janus Neutrons
Ninth Symposium on Microdosimetry Toulouse, France
May 20-24, 1985

Dr. Stephen J. Morewitz

The Role of Microcomputers in Medical Sociology
Annual Meeting of the Midwest Sociological Society, Chicago, IL
April 18, 1984

Medicine, Social Control and Social Change: A Case Study of Michael Reese Hospital and the Jewish Community
Seventy-ninth Annual Meeting of the American Sociological Association, San Antonio, TX
August 27, 1984

Sexual Harassment in the Workplace: Social Control and Social Change
British Sociological Association, War, Violence, and Social Change Conference, Kingston - Upon - Hull, England
April 2, 1985

Dr. Meyrick J. Peak

Reactive Oxygen Species in the Biological Effects of Solar Ultraviolet Radiation
Cosmo '84 Conference on Singlet Oxygen, Clearwater Beach, FL
January 4-7, 1984

Action Spectrum for the Induction of DNA-to-Protein Covalent Cross Links in Human Cell DNA by Ultraviolet Radiation and Blue Light
Ninth International Congress on Photobiology and the Twelfth Annual Meeting of the American Society for Photobiology, Philadelphia, PA
July 1-6, 1984

Molecular Effects of Solar Ultraviolet Radiation
Workshop on UVA, Philadelphia, PA
July 6-7, 1984

Direct and Indirect Mechanisms for the Induction of Apparent DNA-Protein Crosslinks by Far- and Near-Ultraviolet Radiations in Human Cells in Culture
Thirty-third Annual Scientific Meeting of the Radiation Research Society, Los Angeles, CA
May 5-9, 1985

Molecular Photobiology of Solar-UV
Department of Biochemistry, University of California, Berkeley, CA
May 10, 1985

Spectral Analysis of Cytotoxicity, Mutation, and DNA Damages Caused by UVA Radiations

The Effects of Near-UV Radiation on Bacterial and Mammalian Cell DNA
Thirteenth Annual Meeting of the American Society for Photobiology, New Orleans, LA
June 23-27, 1985

Dr. Yueh-Erh Rahman

Liposome Uptake Characteristics of Isolated Liver Parenchymal Cells. Effects of Size, Charge, and Fluidity of Liposomes
Third International Congress in Cell Biology, Tokyo, Japan
August 26-September 1, 1984

Liposomes as Carriers for Targeted Delivery of Metal Chelators
Health Science Center, University of Minnesota, Minnneapolis, MN
April 12, 1985

Endocytic Properties of Normal and Preneoplastic Hepatocytes
Sixty-ninth Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, CA
April 21-26, 1985

Dr. Christopher A. Reilly

Toxicological Evaluation of Complex Organic Mixtures
Abbott Laboratories Seminar, North Chicago, IL
May 18, 1984

Bladder Tumors Resulting from Dermal Exposure to Aromatic Amines
DOE/OHER Complex Mixtures Program Contractors' Meeting, Burr Ridge, IL
June 17-18, 1985

Dr. John Rundo

Long-Term Retention of Radium in Female Former Dial Workers
European Late-Effects Project Symposium on Metals in Bone, Angers, France
October 11-13, 1984

Current (1984) Status of the Study of ^{226}Ra and ^{228}Ra in Humans at the Center for Human Radiobiology

Argonne-Utah Studies of ^{224}Ra Endosteal Surface Dosimetry
Symposium on the Radiobiology of Radium and Thorotrast, Neuherberg, Federal Republic of Germany
October 29-31, 1984

Radon in Man and His Environment
Department of Nuclear Engineering and Radiological Sciences, University of Washington, Seattle, WA
January 21, 1985

Radon in Man and His Environment
Joint Center for Graduate Studies, Richland, WA
January 22, 1985

Current (1984) Status of the Study of ^{226}Ra and ^{228}Ra in Humans at the Center for Human Radiobiology
Biology Department, Battelle-Pacific Northwest Laboratories, Richland, WA
January 22, 1985

Body Radioactivity Measurement in Radiological Protection
Department of Medical Physics, University of Wisconsin, Madison, WI
February 18, 1985

Dr. Marianne Schiffer

Interactions of Immunoglobulin Constant Domains
Gordon Research Conference on Physics and Physical Chemistry of Biopolymers, Plymouth, NH
June 27, 1984

A Model for Secretory Component IgA Interactions
Midwest Autumn Immunology Conference, Chicago, IL
November 3-5, 1984

Structure of Immunoglobulin Light Chains
Department of Biochemistry Seminar, University of Wisconsin Medical School, Milwaukee, WI
November 13, 1984

Dr. Robert A. Schlenker

Dosimetry and Diffusion of ^{220}Rn from Bone Surface Deposits of ^{224}Ra
Annual Meeting of the Radiation Research Society, Orlando, FL
March 27, 1984

Direct Approach to Utilizing Risk Information in Establishing Permissible Levels
Annual Meeting of the National Council on Radiation Protection and Measurements, Washington, DC
April 4, 1984

Autoradiography of the Mastoid Air Cells in Radium Patients
Annual Meeting of the Health Physics Society, New Orleans, LA
June 5, 1984

The Distribution of Radium and Plutonium in Human Bone
European Late-Effects Project Symposium on Metals in Bone, Angers, France
October 11-13, 1984

Argonne-Utah Studies of ^{224}Ra Endosteal Surface Dosimetry
Symposium on the Radiobiology of Radium and Thorotrast, Neuherberg, Federal Republic of Germany
October 30, 1984

The U.S. Radium Study, Health Effects and Dosimetry
Engineering and Radiological Sciences Department, University of Washington, Seattle, WA
January 28, 1985

The U.S. Radium Study, Health Effects and Dosimetry
Joint Center for Graduate Studies, Richland, WA
January 29, 1985

Dosimetry of ^{224}Ra and ^{226}Ra: People to Cells
Biology Department, Battelle Pacific Northwest Laboratories, Richland, WA
January 29, 1985

Theoretical and Experimental Investigation of ^{224}Ra Endosteal Dosimetry
Radiobiology Division, University of Utah, Salt Lake City, UT
March 18, 1985

Dr. Thomas M. Seed

Cellular Changes during Radiation Leukemogenesis
North American Late Effects Group Symposium and Thirty-second Annual Meeting of the Radiation Research Society, Orlando, FL
March 25, 1984

Radiation Effects on Cells and Tissues: Welcome and Introductory Remarks

Induction of Aplastic Anemia by Chronic Low-Dose Gamma Irradiation: Alterations of the Endosteum
Symposium on the Radiation Effects on Cells and Tissues, Annual Meeting of the Scanning Electron Microscopy Society, Philadelphia, PA
April 20, 1984

Radiosensitivity of Hematopoietic Progenitors during Preclinical Phases of Chronic Radiation-Induced Aplastic Anemia and Myeloid Leukemia
Thirteenth Annual Meeting of the International Society for Experimental Hematology, Atlanta, GA
August 12-16, 1984

Cellular Responses in Radiation Leukemogenesis
Symposium on The Role of Chemicals and Radiation in the Etiology of Cancer, Oak Brook, IL
August 28, 1984

Mechanisms of Radiation-Induced Hematopoietic Dysfunction and Its Pathological Sequelae
Workshop on Research Issues in Radiation Late Effects Studies in the Beagle, Argonne National Laboratory, Argonne, IL
November 28-30, 1984

Dr. James H. Stebbings

Erythrocytic Leukemia as a Sex-Determined Response to Chronic Irradiation
Twenty-ninth Annual Meeting of the Health Physics Society, New Orleans, LA
June 3-8, 1984

Epidemiologic Aspects of Risk Estimation for Cancer Induced by Radium in Man
American Statistical Association Conference on Radiation and Health (Coolfont IV), Coolfont, WV
July 8-13, 1984

Health Effects of Radium
Hannah City Village Board Meeting, Hannah City, IL
January 15, 19[illegible]

Times-to-Tumor of Radium-induced Sarcomas and Carcinomas in Humans
NCI/DOE Symposium on Time-related Factors in Cancer Epidemiology, National Institute of Health, Bethesda, MD
April 15-17, 1985

Dr. John Taylor

Design Specifications for a Graphic system for the Analysis of Sets of Proteins
Third International Symposium on Two-Dimensional Electrophoresis Protein Mapping
June 10-14, 1984

Mr. David V. Tolle

Hematology and Pathology of Radiation-Induced Myeloproliferative Disease
Workshop on Research Issues in Radiation Late Effects Studies in the Beagle, Argonne National Laboratory, Argonne, IL
November 28-30, 1984

Dr. Richard E. Toohey

Radiation Exposure
Roundtable on Low-Level Contamination and Outpatient Treatment, Foundation for Advancements in Science and Education, Los Angeles, CA
May 11, 1984

An Unusual Case of Radium Exposure
Twenty-ninth Annual Meeting of the Health Physics Society, New Orleans, LA
June 3-8, 1984

Radium in Drinking Water
Kane County Water Works Association,
October 25, 1984

Activity Ratios of Thorium Daughters In Vivo
Symposium on the Radiobiology of Radium and Thorotrast, Neuherberg, Federal Republic of Germany
October 29-31, 1984

Radon Levels in Single-Family Houses
Midyear Symposium of the Health Physics Society, Colorado Springs, CO
January 6-10, 1985

Health Effects of Radium
Illinois Groundwater Association, Morris, IL
April 18, 1985

In Vivo Monitoring Capabilities for Transuranics
Thirtieth Annual Meeting of the Health Physics Society, Chicago, IL
May 26-31, 1985

Whole-Body Counting, Emergency Preparedness and Response
Health Physics Society Summer School, Evanston, IL
June 4, 1985

The Health Effects of Radium - Observed and Assumed
Testimony presented before the Illinois Pollution Control Board, Aurora, IL
June 25, 1985

Dr. Robert L. Wells

Action Spectra for Killing and Mutation of Chinese Hamster Cells Exposed to Mid- and Near-Ultraviolet Monochromatic Light
Thirty-second Annual Meeting of the Radiation Research Society, Orlando, FL
March 25-29, 1984

Dr. Edwin M. Westbrook

The 2.5 Å Structure of Δ^5-3-Ketosteroid Isomerase
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT
May 3, 1985

Biomedical Applications of Synchrotron Radiation
6 GeV Synchrotron Workshop, Chicago, IL
May 16, 1985

The 2.5 Å Structure of Δ^5-3-Ketosteroid Isomerase
Laboratory for Molecular Pharmacology, Johns Hopkins University Medical School, Baltimore, MD
May 17, 1985

26. PROFESSIONAL AFFILIATIONS

Joint and Adjunct College or University Appointments
Other Professional Activities

Joint and Adjunct College or University Appointments

Sixteen staff members held a total of 19 faculty appointments at seven different colleges and universities. These appointments usually require limited teaching activities at the graduate level, generally of a specialized nature, and involve regular contact with students. These appointments have often led to co-sponsorship of graduate students and to collaborative efforts with faculty members, some of which are described in this report.

Norman G. Anderson, Adjunct Professor, Department of Surgery, Medical University of South Carolina, Charleston, SC, 1974-present

Maryka H. Bhattacharyya, Adjunct Clinical Professor, Departments of General and Oral Pathology, Loyola University, Chicago, IL, 1979-present

Maryka H. Bhattacharyya, Adjunct Assistant Professor, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, 1979-present

Thomas J. Doody, Lecturer, Chemistry Department, Illinois Benedictine College, Lisle, IL, 1963-present

Thomas E. Fritz, Clinical Associate Professor, Department of Pathology, Loyola University, Chicago, IL, 1975-present

Thomas E. Fritz, Adjunct Associate Professor, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, 1978-present

Douglas Grahn, Adjunct Professor, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, 1971-present

David J. Grdina, Visiting Associate Professor, Department of Therapeutic Radiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, April 1984-January, 1985

David J. Grdina, Associate Professor, Department of Therapeutic Radiology, University of Chicago, Chicago, IL, February 1985-present

Colin K. Hill, Instructor, Department of Therapeutic Radiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL, 1981-present

Eliezer Huberman, Professor, Departments of Molecular Genetics and Cell Biology and Therapeutic Radiology, University of Chicago, Chicago, IL, 1982-present

Walter E. Kisieleski, Clinical Professor, Departments of Radiology and Oral Pathology, Loyola University Medical and Dental Schools, Chicago, IL, 1973-present

Herbert E. Kubitschek, Adjunct Professor, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, 1971-present

Meyrick J. Peak, Adjunct Professor, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, 1982-present

Carl Peraino, Adjunct Professor, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, 1971-present

Yueh-Erh Rahman, Adjunct Professor, Graduate School of Arts and Sciences, Northern Illinois University, DeKalb, IL, 1971-present

Christopher A. Reilly, Adjunct Associate Professor, Department of Biological Sciences, Northern Illinois University, DeKalb, IL, 1975-present

Marianne Schiffer, Lecturer of Biochemistry, Molecular Biology, and Cellular Biology, Northwestern University, Evanston, IL, 1983-present

Edwin J. Westbrook, Assistant Professor, Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, IL, 1983-present

Other Professional Activities

Staff members from the Division were well represented as members of editorial boards or advisory bodies or in positions of leadership in professional societies. Twelve of the Division staff members participated in these activities as detailed below:

Members of Editorial Boards

Norman G. Anderson, Member of Editorial Board, Molecular Biology and Medicine

N. Leigh Anderson, Member of Editorial Board, Electrophoresis

Charles F. Ehret, Consulting Editor, International Journal of Chronobiology

Charles F. Ehret, Section Editor for Biology, Journal of the URAM Institute (Institute for Encyclopedia of Human Ideas on Ultimate Reality and Meaning, Toronto)

Eliezer Huberman, Associate Editor, Cancer Research

Eliezer Huberman, Member of Editorial Board, Teratogenesis, Carcinogenesis, and Mutagenesis Journal

Herbert E. Kubitschek, Guest Editor, Molecular and Cellular Biophysics

Carl Peraino, Associate Editor, Cancer Research

Thomas M. Seed, Guest Editor, International Journal of Scanning Electron Microscopy

Members of Advisory or Review Bodies

Charles F. Ehret, Member, International Scientific Advisory Board, Center for Design of Industrial Schedules

Thomas E. Fritz, Member, National Council on Radiation Protection and Measurements Scientific Committee 57, Task Group on Leukemia Risk

Thomas E. Fritz, Board of Directors, Illinois Cancer Council

Douglas Grahn, COSPAR, Ordinary Member, Sub-Commission F-2 on Radiation Biology of Interdisciplinary Scientific Commission F on Life Sciences as Related to Space

Douglas Grahn, Member, National Council on Radiation Protection and Measurement Committee 57, Internal Emitter Standards, Task Group 11, Genetic Risk

Douglas Grahn, Member, National Council on Radiation Protection and Measurement Committee 75, Guidance on Radiation Received in Space Activities

Eliezer Huberman, Member, National Institute of Environmental Health Sciences Review Committee

Eliezer Huberman, Member, Committee on Genetics, University of Chicago

Louise Lombard, Member, Board of Scientific Counselors of the Division of Cancer Cause and Prevention, National Cancer Institute

Yueh-Erh Rahman, Member, Committee of Review Group, Division of Research Grants, National Institutes of Health

Officers or Board Members of Professional/Scientific Associations

Charles F. Ehret, Vice President, International Society for Chronobiology

Charles F. Ehret, Chairman, Committee on Chronobiological Engineering, International Society for Chronobiology

David J. Grdina, Chairman, Finance Committee, Radiation Research Society

Meyrick J. Peak, Councilor, American Society for Photobiology

27. PUBLICATIONS

Journal Articles

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Au, W. W., M. F. Callaham, M. L. Workman, and E. Huberman
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Baldwin, W. W., and H. E. Kubitschek
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Repair Processes in Radiation-Induced Transformation
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J. Mol. Biol. 173, 109-123 (1984)

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Cell Tissue Kinet. 17, 223-236 (1984)

Grdina, D. J., H. D. Thames, and L. Milas
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Han, A., C. K. Hill, and M. M. Elkind
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Han, A., M. J. Peak, and J. G. Peak
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Hill, C. K., A. Han, and M. M. Elkind
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Jones, C. A., M. F. Callaham, and E. Huberman
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28. STAFF AND FUNDING AGENCIES*

CARCINOGENESIS SECTION

Hepatocarcinogenesis

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DOE: Carcinogenesis (HA-02-02-01) ANL-60400 "Control of Gene Expression in Normal and Neoplastic Liver"

NIEHS: Interagency Agreement, (Y01-ES-20091) ANL-8D410 "Refinement and Use of Peraino Rat Liver Tumor Model"

*Staff listings and funding agencies shown cover the period January 1, 1984, through June 1985. Participating staff members from ANL divisions other than the Division of Biological and Medical Research are not included.

The individual groups are arranged in the order in which the program summaries are presented in this report. Group titles in this listing are those in the organizational chart (p. ix).

Abbreviations: DOE, Department of Energy; NCI, National Cancer Institute; NRC, Nuclear Regulatory Commission; NIADDK, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases; NIAID, National Institute of Allergy and Infectious Diseases; NIEHS, National Institute of Environmental Health Sciences.

Metal Carcinogenesis

Regular Staff

Y. E. Rahman (Senior Biologist)
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DOE: Carcinogenesis (HA-02-02-01)
ANL-60414 "The Role of Metals as Cocarcinogens"

NIADDK: 5 RO1 AM21592
"New Iron Chelator Delivery System for Cooley's Anemia"
(Project completed 03/03/84.)

Gene Expression and Carcinogenesis

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M. Stodolsky (Visiting Scientist)

DOE: Human Health Effects from Energy Generation (HA-02-01-01)
ANL-63304 "Oncogenes and Human Radium Carcinogenesis"

DOE: Carcinogenesis (HA-02-02-01)
ANL-60417 "Chemical Carcinogenesis Studies in Human and Rodent Cells"

NIEHS: Interagency Agreement (Y01-ES-20102) ANL-8D408
"Transformation of Syrian Hamster Embryo Cells by Carcinogenic Chemicals"

EPA: Interagency Agreement (DW930217-01) ANL-8H458
"Development and Use of a Human Teratoma Cell Culture for the Prescreening of Environmental Chemicals which May Initiate or Promote Tumor Formation"

Mammalian Cell Biology

Regular Staff

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DOE: Mutagenesis (HA-02-02-02)
ANL-61300 "Radiation-Induced Changes in Mammalian Cells"

NCI: 1 RO1 CA33974
"Mutation-Transformation: Neutron Damage and Repair"

HUMAN RADIOBIOLOGY SECTION

Radionuclide Metabolism

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R. A. Schlenker (Biophysicist)
R. E. Toohey (Biophysicist)

M. A. Essling (Scientific Assoc.)
F. Markun (Scientific Assoc.)

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DOE: Human Health Effects from Energy Generation
(HA-02-01-01)
ANL-63300 "Effects of Internally Deposited Alpha Emitters"

NRC: Div. of Health, Siting, and Waste Management
ANL-8M440 "Health Effects of Industrial Exposure to Thorium"

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DOE: Human Health Effects from Energy Generation
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ANL-63300 "Effects of Internally Deposited Alpha Emitters"

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NRC: Div. of Health, Siting, and Waste Management
ANL-8M440 "Health Effects of Industrial Exposure to Thorium"

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NRC: Div. of Health, Siting, and Waste Management
ANL-8M628 "Lung Cancer in Women Occupationally Exposed to Radon in Early Adult Life"

Measurements

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DOE: Human Health Effects from Energy Generation
(HA-02-01-01)
ANL-63300 "Effects of Internally Deposited Alpha Emitters"

NRC: Div. of Health, Siting, and Waste Management
ANL-8M440 "Health Effects of Industrial Exposure to Thorium"

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DOE: Analytical Studies
(HA-02-04-01)
ANL-67505 "Assessment of Radon and Radon Daughters in U. S. Single-Family Housing"

Dosimetry and Tumor Mechanisms

Regular Staff

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DOE: Human Health Effects from Energy Generation
(HA-02-01-01)
ANL-63300 "Effects of Internally Deposited Alpha Emitters"

DOE: Carcinogenesis
(HA-02-02-01)
ANL-63205 "Tumor Models and Mechanisms"

LOW-LEVEL RADIATION SECTION

Radiation Carcinogenesis

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DOE: Carcinogenesis (HA-02-02-01)
ANL-60300 "Life Shortening and Tumor Induction by Low Dose Neutron and Gamma Irradiation"

NCI: 7R01 CA37435
ANL-85414 "Radiation Response of Cells Separated from Tumors"

Radiation Biology and Genetics

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DOE: Carcinogenesis (HA-02-02-01)
ANL-60300 "Life Shortening and Tumor Induction by Low Dose Neutron and Gamma Irradiation"

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

B. F. Farrington (Scientific Asst.)
C. A. Fox (Programmer)
G. L. Holmblad (Scientific Assoc.)
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V. A. Ludeman (Scientific Asst.)
A. R. Sallese (Scientific Asst.)
E. F. Staffeldt (Scientific Assoc.)
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NRC: Div. of Health, Siting, and Waste Management
ANL-8M456 "Relative Biological Effectiveness of Fission Neutrons and Gamma Rays at Occupational Exposure Levels"

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Radiation Toxicology

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G. L. Holmblad (Scientific Assoc.)
L. V. Kaspar (Scientific Assoc.)
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A. R. Sallese (Scientific Asst.)

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

NCI: Interagency Agreement (Y01-CO-0320) ANL-8D407 "Late Effects of Protracted Irradiation in Dogs"

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Radiation Hematology

Regular Staff T. M. Seed (Biologist) Principal Investigator L. V. Kaspar (Scientific Assoc.)	DOE:	Carcinogenesis (HA-02-02-01) ANL-63105 "Hematopoiesis in Chronic Toxicity Studies"

MOLECULAR BIOLOGY SECTION

Photobiology

Regular Staff M. J. Peak (Microbiologist) Principal Investigator J. G. Peak (Asst. Biologist)	DOE:	Mutagenesis (HA-02-02-02) ANL-62201 "Cellular and Molecular Mechanisms of Radiation and Chemical Mutagenesis"
Temporary Staff A. Ito (Postdoc. Appointee)	NCI	5 R01 CA34492 "Solar UV Damage in Human Cells"
	NCI	5 R01 CA37848 "Biological Effects of Solar-UV-Generated Oxygen Species"

Molecular Anatomy

Regular Staff N. G. Anderson (Sr. Physiologist) Principal Investigator N. L. Anderson (Biophysicist) C. S. Giometti (Asst. Biologist) J. Taylor (Computer Scientist) M. A. Gemmell (Scientific Assoc.) S. L. Nance (Scientific Assoc.) S. L. Tollaksen (Scientific Assoc.)	DOE:	Systems Damage (HA-02-02-03) ANL-61203 "Identification of Human Proteins with Two-Dimensional Electrophoresis"
	DOE:	Human Health Effects from Energy Generation (HA-02-01-01) ANL-68112 "Development of New Technologies for Human Health Effects Studies"

Molecular Biophysics

Regular Staff

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DOE: Systems Damage (HA-02-02-02)
ANL-61200 "Protein Structure and Conformation"

NIAID: 5 R01 AI19590
"Study of Anti-Idiotypic Antibodies"

NIAID: 1 R01 AI21695
"Crystallographic Studies of Neutrophil Cationic Proteins"

Mutagenesis

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D. Williams-Hill (Scientific Asst.)

DOE: Mutagenesis (HA-02-02-02)
ANL-62201 "Cellular and Molecular Mechanisms of Radiation and Chemical Mutagenesis"

NIAID 1 R01 AI21954
"Growth during the Cell Cycle"

TOXICOLOGY SECTION

Chemical Toxicology

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Temporary Staff

M. L. Cunningham (Postdoc. Appointee)

DOE: Carcinogenesis (HA-02-02-01) ANL-60404 "Toxicology of Complex Mixtures of Organic Compounds"

Mutagenesis (HA-02-02-02) ANL-62200 "Cellular Toxicology of Complex Mixtures of Organic Compounds"

DOE: Fossil Energy (AA-85-50-05) ANL-49533 "Health and Environmental Characterization of Coal Gasification Materials"

Chemical and Biological Characterization

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Temporary Staff

M. S. Swanson (Postdoc. Appointee)

DOE: Analytical Studies (HA-02-04-01) ANL-60407 "Chemical Characterization of Toxicologically Significant Complex Mixtures of Organic Compounds"

Metal Toxicology

Regular Staff M. H. Bhattacharyya (Biochemist) Principal Investigator	DOE:	Systems Damage (HA-02-02-03) ANL-61209 "Biological Effects of Metals"
E. S. Moretti (Scientific Asst.) D. P. Peterson (Scientific Asst.)	DOE:	Systems Damage (HA-02-02-03) ANL-61213 "Gastrointestinal Absorption of Actinides"
Temporary Staff B. D. Whelton (Scientist in Residence)	NRC:	Div. of Health, Siting, and Waste Management ANL-8M447 "Reanalysis of Gastrointestinal Absorption Factors for Plutonium and Other Actinide Elements"

Neurobehavioral Chronobiology

Regular Staff C. F. Ehret (Sr. Biologist) Principal Investigator	DOE:	Systems Damage (HA-02-02-03) ANL-61208 "Neurobehavioral Chronobiology and Toxicology"
K. R. Groh (Scientific Assoc.) Temporary Staff D. J. Fowler (Scientist in Residence)	DOE:	Electric Energy Systems (AK-05-02) ANL-49185 "Analysis of How and Why 60-Hz Electric Fields Cause Phase Shifts and Dyschronism in Small Rodents"

AUTHOR INDEX

Ainsworth, C. F. 89
Anast, G. A. 27, 35
Anderson, N. G. 81
Anderson, N. L. 81
Angerman, J. M. 49
Bedekar, S. 89
Bhattacharyya, M. H. 109
Blomquist, J. A. 3
Boparai, A. S. 105
Buess, E. M. 19
Burcham, J. M. 3
Callaham, M. F. 11
Carnes, B. A. 3, 59, 63
Cerny, E. A. 7
Chang, C. H. 89
Chien, F. 11
Collart, F. 11
Cullen, S. M. 63
Dale, P. J. 49
Doyle, D. E. 63
Duggal, K. 53, 63
Ehret, C. F. 115
Elkind, M. M. 19
Essling, M. A. 27, 35, 39
Farnham, J. E. 27
Farrington, B. H. 53, 59
Fowler, D. J. 115
Fritz, T. E. 63
Gemmell, M. A. 81
Giometti, C. S. 81
Grahn, D. 53
Grdina, D. J. 49
Groh, K. R. 115
Han, A. 19
Hanly, C. W. 89
Hardwick, J. P. 11
Haugen, D. A. 105
Heffley, P. D. 105
Henning, C. B. 11
Hill, C. K. 19
Hofmann, J.-P. 81
Holmblad, G. L. 53, 63
Homma, Y. 11
Huberman, E. 11
Hulesch, J. L. 53
Ito, A. 75
Jonah, M. M. 7
Jones, C. A. 11
Kaspar, L. V. 63, 69
Keane, A. T. 27
Keenan, W. G. 63
Kiguchi, K. 11
Knasko, G. L. 27
Kokaisl, G. A. 27
Kotek, T. J. 27, 35
Kubitschek, H. E. 95
Lagocki, P. A. 7
Larsen, R. P. 109
Lee, C.-H. 59
Liu, C.-M. 19
Lombard, L. S. 53, 63
Lucas, H. F. 27, 35
Ludeman, V. A. 3, 53
Maeda, S. 11
Markun, F. 27, 35, 39
Marshall, I. R. 53
Moretti, E. S. 109
Murison, G. L. 11
Nagy, B. 49
Naik, V. 89
Nance, S. L. 81
Oldham, R. D. 27, 109
Oltman, B. A. 27
Oltman, B. G. 43
Oravec, C. T. 11
Peak, J. G. 75
Peak, M. J. 75
Peraino, C. 3
Peterson, D. P. 109
Poole, C. M. 63
Prapuolenis, A. M. 3
Qian, F. 11
Rahman, Y. E. 7
Reilly, C. A., Jr. 101
Roth, R. M. 75
Rundo, J. 27, 35
Russell, J. J. 3
Sallese, A. R. 53, 63
Schiffer, M. 89
Schlenker, R. A. 27, 43
Sedita, D. A. 11
Seed, T. M. 63, 69

Staffeldt, E. F. 3, 53
Stamoudis, V. C. 105
Stebbings, J. H. 27, 35
Stevens, F. J. 89
Stewart, J. 105
Stodolsky, M. 11
Suhrbier, K. M. 105
Swanson, M. S. 105
Taylor, J. 81
Thompson, E. G. 27, 43
Thomson, J. F. 53
Tollaksen, S. L. 81
Tolle, D. V. 63
Toohey, R. E. 27, 35, 39
Trier, J. E. 53
Vesselinovitch, S. D. 3
Wardrip, C. 63
Wells, R. L. 19
Westbrook, E. M. 89
Westholm, F. A. 89
Whelton, B. D. 109
Williamson, F. S. 53
Wright, B. J. 53
Young, T. S. 89

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