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Division of Biological and Medical Research

Annual Research Summary

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E. Huberman, Division Director
S. H. Barr, Editor

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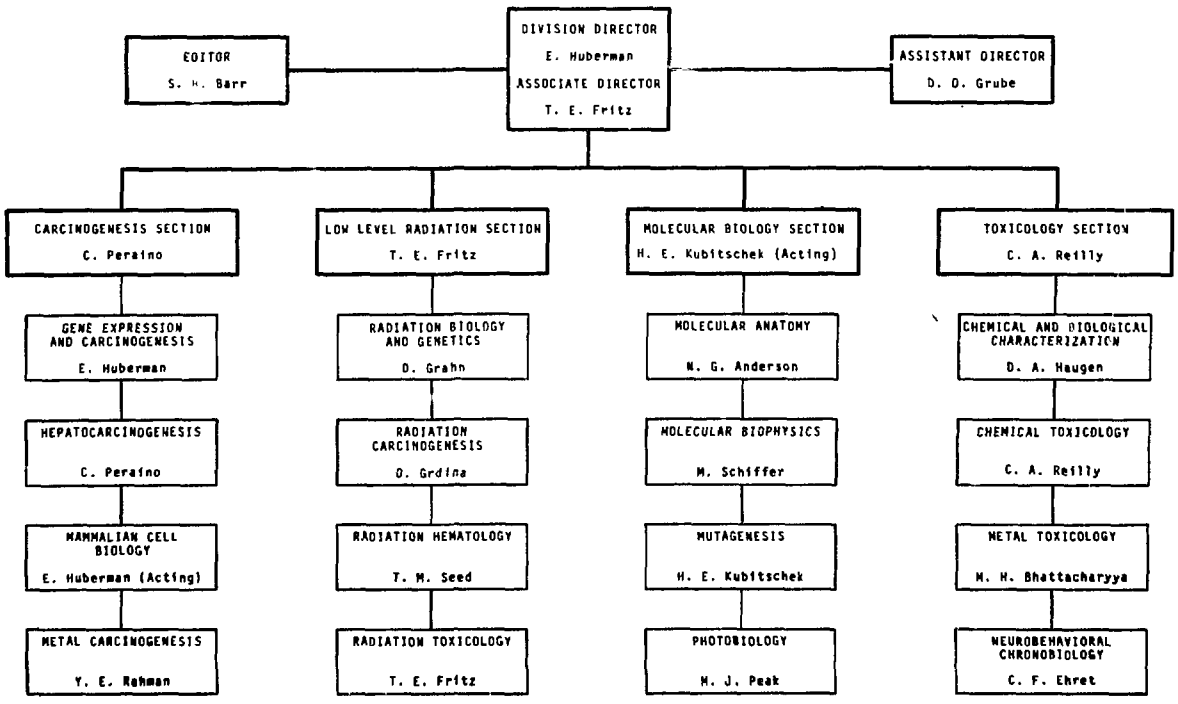
The building shown below houses the Division of Biological and Medical Research. This multipurpose facility is located on Inner Circle Drive at Argonne National Laboratory's main site, which occupies 1,700 acres about 25 miles southwest of Chicago.

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, and environmental toxicology. 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, toxicologists, veterinarians, statisticians, computer analysts, and engineering specialists.

The Division is organized into four scientific sections: Carcinogenesis, Low Level Radiation, Molecular Biology, and Toxicology. Each section comprises four groups, as illustrated in the following organizational chart. The sectional organization emphasizes broad scientific relationships and encourages interactions among staff members with related interests. Each section holds regular seminars, which persons from other sections are encouraged to attend.



This research summary is organized into five parts. The first four parts reflect the Divisional structure and contain the scientific program chapters, which summarize the activities of the individual groups during the calendar year 1983 and the first half of 1984. To provide better continuity and perspective, previous work is sometimes briefly described. Because the summaries have been kept short, they do not necessarily describe all the work of a research group. Each of the scientific chapters is followed by a list of relevant publications of the staff members that were received in 1983 and the first half of 1984. Also included are publications that were accepted for publication as of June 30, 1984.

The final part of this report contains chapters that indicate the range of other activities in the Division. They describe the animal and radiation 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 1983, 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--for the calendar year 1983. The last chapter presents the staff and the governmental agencies supporting each of the Divisional groups.

This year BIM staff members were honored for a variety of accomplishments. Dr. Carl Peraino received a 1983 University of Chicago Award for Distinguished Performance at Argonne National Laboratory for his model of multistage carcinogenesis in the rat liver. Dr. Mortimer Elkind received a Gold Medal for Distinguished Achievement from the American Society of Therapeutic Radiologists for scientific contributions in basic radiobiology and his landmark work on repair of sublethal x-ray damage of cells. In addition, he was awarded the 1984 G. Failla Memorial Award by the Radiation Research Society for significant achievements in radiation research and the 1984 Albert Soiland Memorial Award by the Southern California Cancer Center for his work on the repair of radiation damage and its influence on the advancement of radiation therapy. The Gerontological Society of America dedicated several of the symposia at its 1983 meeting to the memory of George Sacher and established the George A. Sacher Award for the best presentation by a student biologist at the annual meeting of the Society. Dr. Norman G. Anderson was recognized by Argonne National Laboratory with the Career Patent Leader Award for his more than 20 patents associated with the molecular anatomy program. Dr. Charles Ehret received the Federal Laboratory Consortium 1984 Special Award for Excellence in Technology Transfer, which recognizes special creativity and initiative and dedication to the scientific profession above and beyond institutional responsibilities. His award citation highlighted his research in circadian regulation and its application to

the design of methods to eliminate jet lag and to provide safer shift work schedules. Finally, Dr. Charles Borso received an IR-100 Award for his real time synchrotron x-ray small angle scattering instrumentation.

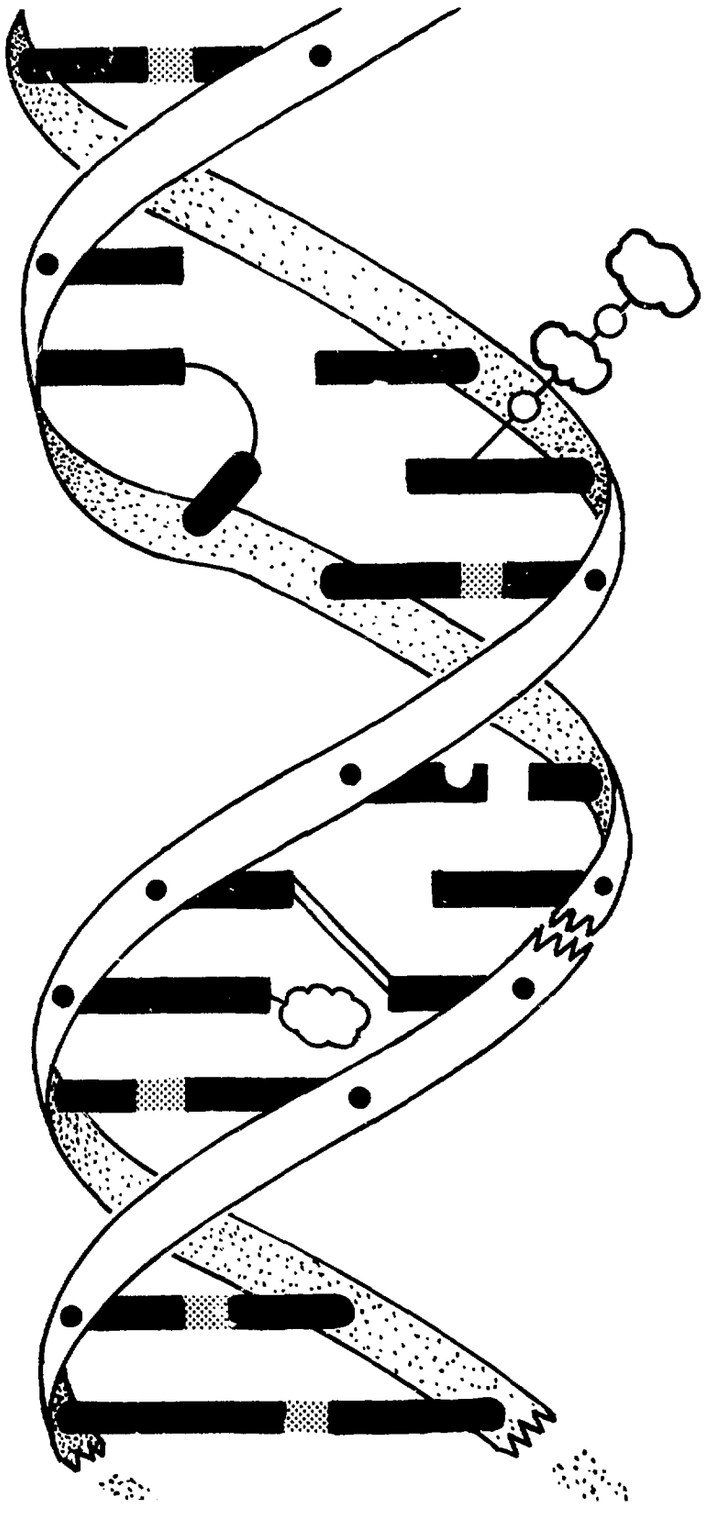
Division staff members have been active this year in the organization of international symposia and programs. Dr. Thomas M. Seed was one of the three organizers of the program, "The Ultrastructure of Radiation-Induced Injury in Cells and Tissues," which took place during the Scanning Electron Microscopy meeting in Philadelphia on April 20, 1984. The highly successful Third International Symposium on Two-Dimensional Electrophoresis Protein Mapping, organized by Sandra L. Tollaksen, Dr. Norman G. Anderson, and Dr. Leigh Anderson, was held at Argonne from June 10 to 14, 1984. Finally, 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, which I chaired, consisting of Drs. Thomas E. Fritz, Colin K. Hill, Carol A. Jones, and Carl Peraino. This symposium, which was held at the Hyatt Oak Brook Hotel, Oak Brook, Illinois, on August 26-29, 1984, was co-sponsored by Argonne, the University of Chicago, the U. S. Department of Energy, the U. S. Environmental Protection Agency, Hoffman-La Roche, Inc., and the Burroughs Wellcome Co. The proceedings of this symposium will be published by Raven Press.

To conclude on a sober note, we will miss Dr. Tony Han, who died suddenly on May 7, 1984. Dr. Han, who held concurrent appointments as a biophysicist in the Mammalian Cell Biology Group of our Division and as a professor of Radiation Oncology and Director of the Cancer Research Laboratory, University of Southern California School of Medicine, contributed significantly to the advancement of knowledge in the areas of radiation-induced damage to DNA and radiation-induced neoplastic transformation. Dr. Han was also an active member of several Argonne committees, an Associate Editor of Radiation Research, and a member of the Board of Editors of the International Journal of Radiation Biology.

E. Huberman

E. Huberman, Director
Division of Biological
and Medical Research

Carcinogenesis



In the photograph below, Betty Jean Wright is using an image digitizer to assess preneoplastic changes in histochemically stained sections of rat liver. See Chapter 1 for a description of the major program in which this technique is used.

1. MECHANISMS OF HEPATOCARCINOGENESIS

C. Peraino, Principal Investigator
B. A. Carnes,* J. M. Burcham, W. E. Boernke,† J. E. Morris,§
J. A. Blomquist, V. A. Ludeman, A. M. Prapuolenis,
E. F. Staffeldt, and J. J. Russell

The program objectives are (1) to determine the mechanisms of liver tumor initiation and promotion and (2) to further refine the experimental liver tumorigenesis system developed in our laboratory as a rapid bioassay for carcinogens. We are using two complementary experimental approaches to examine the morphological and biochemical characteristics of hepatocytes undergoing tumorigenesis and the nature of the gene control processes that are functionally linked to the expression of neoplasia.

Our tumorigenesis studies involve the administration of presumptive tumor initiators [e.g., diethylnitrosamine (DEN) or benzo(a)pyrene (BAP)] to newborn rats followed by the feeding of presumptive promoters (e.g., phenobarbital) beginning at the time of weaning. Histochemical analysis of the liver as early as six weeks after carcinogen treatment reveals the formation of small aggregations of hepatocytes (foci) with biochemical changes like those subsequently appearing in most liver tumors. Foci and tumor incidences and characteristics are then compared. The sensitivity, rapidity, and simplicity of this new treatment protocol have enhanced the ability of the liver system to detect environmental carcinogens and are facilitating the analysis of the critical changes in liver cells associated with the onset of neoplasia.

Our gene control studies involve comparisons of the mechanisms controlling the synthesis of two enzymes, serine dehydratase (SDH) and ornithine aminotransferase (OAT), which undergo adaptive changes in tumors. Following dietary, hormonal, and chronobiotic stimuli, we measure changes in enzyme synthesis rates, functional mRNA levels, and transcribable gene content to determine the sites of action of these stimuli. Acquisition of such informa-

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†Visiting Scientist, Nebraska Wesleyan University.
§Scientist in Residence (Faculty Research Leave at Argonne Appointee), State University College of New York, Brockport.

tion is essential for an understanding of gene control mechanisms in normal liver; this knowledge can then serve as the basis for characterizing perturbations in the regulation of gene expression during carcinogenesis.

The following were the principal findings in our tumorigenesis studies: (1) Both the DEN and BAP treatments were tumorigenic and produced foci with similar phenotypic properties (numbers and identities of histochemical markers). (2) Foci relative growth rates and growth capacities (ranges of possible growth rates) were directly related to foci phenotypic complexity levels (numbers of markers per focus). (3) Individual foci were phenotypically stable (i.e., they neither gained nor lost markers). (4) A substantial fraction of the tumors observed in this study had fewer markers than the most complex foci.

In our latest studies of the regulation of SDH and OAT gene expression in normal liver, we examined the circadian cycling characteristics of the rates of enzyme synthesis in rats fed a 60% casein diet for 1 h at 11 h intervals vs. 2 h at 22 h intervals and in rats fed a 20% casein diet vs. a 60% casein diet according to the 2:22 feeding schedule. The data demonstrated that changes in feeding frequency or dietary composition did not abolish the circadian synthesis cycle for either enzyme but that the phasing of the cycle for each enzyme could be shifted independently. These data indicate that the circadian fluctuations in OAT and SDH synthesis are generated by separate endogenous circadian oscillators that influence one or more of the stages of gene expression (transcription, mRNA processing, translation).

Considered collectively, the results of our studies of foci and tumor formation suggest that foci emerge as the result of a specific set of cellular changes solely inducible by carcinogenic stimuli, but that the foci do not eventually become tumors through a process that involves progressive increases in the complexity of the foci. Instead, we postulate that tumorigenesis involves a separate transformation event that may occur in a susceptible fraction of foci subsequent to their formation or, alternatively, may occur at the time of exposure to carcinogen, in parallel with the carcinogen-mediated events leading to focus formation. Analysis of the mechanisms underlying the disruptions in gene expression involved in the production of foci and tumors will be based on techniques and insights gained in our studies of SDH and OAT gene regulation.

PUBLICATIONS APPEARING DURING THE NOTED PERIODS

1983

Peraino, C., W. L. Richards, and F. J. Stevens
Multistage Hepatocarcinogenesis
Mechanisms of Tumor Promotion, Vol. I, Tumor Promotion in Internal Organs, T. J. Slaga, ed., CRC Press, Inc., Boca Raton, FL, 1983, Chap. 1, pp. 1-53

Stevens, F. J., and C. Peraino
Liver as a Model System for Analyzing Mechanisms of Tumor Initiation and Promotion
Organ and Species Specificity in Chemical Carcinogenesis, R. Langenbach, S. Nesnow, and J. M. Rice, eds., Plenum Publishing Corporation, New York, NY, 1983, pp. 231-252

Accepted as of June 30, 1984

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 the Symposium on the Role of Cocarcinogens and Promoters in Human and Experimental Carcinogenesis, Budapest, Hungary, 5/16-18/83

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.

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

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

The objectives of this program are (1) to establish and develop animal models to investigate metal tumorigenesis, (2) to define the specific role of metal compounds in the process of tumor induction, and (3) to develop new approaches for reducing the tumorigenicity and toxicity of the metals under investigation.

Our studies on the role of metals in the induction of tumors currently focus on arsenic. To study carcinogenesis of the skin, we gave mice arsenic at different levels in their drinking water, first in conjunction with a tumor initiator (physical or chemical) and then with applications of a skin tumor promoter, 12-O-tetradecanoyl phorbol - 13 - acetate (TPA). The mice were then monitored for the development of skin tumors. In other studies focusing on hepatocarcinogenesis, rats were similarly exposed to arsenic in the drinking water, then injected with a hepatocarcinogen and fed phenobarbital as a promoter. The livers were examined histochemically at intervals for the development of preneoplastic foci.

Using ultraviolet (UV) radiation as the tumor initiator, we had previously demonstrated an enhancing effect of arsenic on the induction of skin tumors. Now, with the use of a chemical carcinogen, 7,12-dimethylbenz[a]anthracene (DMBA), we have further confirmed our findings from the UV experiment. Results obtained from the DMBA experiment are summarized as follows: (1) Among all experimental groups (DMBA alone, arsenic alone, TPA alone, TPA + arsenic, DMBA + arsenic, DMBA + TPA, and DMBA + TPA + arsenic), only the group of mice treated with DMBA + TPA + arsenic showed an increased mortality from all causes. (2) A significantly larger number of papillomas was observed in the group of mice given DMBA + TPA + arsenic over the number of tumors found in the group given only DMBA + TPA. All other experimental groups had only negligible numbers of papillomas. (3) By ~ 22 weeks after the last TPA application, about 5-7% of the papillomas had

*Present Affiliation: Abbott Laboratories, North Chicago, Illinois.

begun to assume the morphology of squamous cell carcinomas. (4) In the group given DMBA + TPA + arsenic, 100% of the mice had skin papillomas by one week after the last TPA application, while in the group given only DMBA + TPA a maximum of 95% of the mice had papillomas at four weeks after the last TPA application.

Analysis of the data from the experiments involving the induction of preneoplastic foci and tumors in the livers of rats, in response to arsenic given in drinking water, has been continued. We have examined liver sections from rats at 9 months after carcinogen administration. The results show that arsenic at a nontoxic level of 10 mg/L has an enhancing effect on the induction of preneoplastic foci in the liver of female but not male rats. We have also performed a similar experiment giving selenium instead of arsenic in drinking water and have found that selenium at a level of 4 ppm has a marked inhibitory effect on the induction of preneoplastic foci in the livers of both female and male rats.

One of the characteristics of the preneoplastic foci of the liver is resistance to iron accumulation. By injecting carcinogen-treated rats with multiple doses of iron-dextran, we obtain specific iron loading in normal hepatocytes while sparing the preneoplastic cells. A Percoll (a self-generating gradient medium) gradient of appropriate densities has been successfully developed to separate the cells of the preneoplastic foci (which are less dense because they lack iron) from the denser normal hepatocytes. Preliminary study of these preneoplastic liver cells indicates that they have an average diameter of about 20 μm, while the average diameter of the normal hepatocytes is about 15 μm.

In the development of a new approach to mobilize toxic metals from storage organs of animals, we used two methods to produce experimental iron overloading in mice: (1) Iron overloading in the reticuloendothelial system was produced by administration of heat-damaged ^{59}Fe-labeled red blood cells and (2) iron overloading in the liver parenchymal cells was produced by administration of purified ^{59}Fe-labeled ferritin. Lipid vesicles (liposomes) differing in size and lipid composition were used to encapsulate various metal chelators for delivery to specific metal storage organs. Short-term cultures of liver parenchymal cells and peritoneal macrophages were also used for in vitro studies of liposome-cell interactions. Results obtained from cultured peritoneal macrophages were reported in last year's report, ANL-83-40.

This year we have further confirmed our previous findings that liver parenchymal cells are capable of endocytic activities. Using various types of liposomes, we observed the following:

1. Given the same surface charge, large unilamellar liposomes (LUV) were more effectively taken up than the small unilamellar liposomes (SUV).

2. Increasing the positive charge of liposomes (by increasing the stearylamine content) enhanced the liposome uptake.

3. For liposomes of the same size, positive liposomes were taken up best, then negative ones, with the lowest uptake for neutral liposomes.

4. Hydrogenation of egg phosphatidylcholine (PC) drastically enhanced the uptake of liposomes regardless of their surface charge.

5. Cytochalasin B, a known inhibitor of phagocytosis, did not significantly affect the uptake of LUV or SUV.

6. Incubation of the liver cells at 4°C, however, resulted in a more than 90% reduction in liposome uptake.

Few studies have been published that focus on the endocytic capability of liver parenchymal cells. Our studies, which include electron microscopic examination, have indicated that these cells can internalize particles of the size of LUV (~ 160 nm). Reducing the fluidity of the liposomal membranes by using hydrogenated PC greatly enhanced the liposome uptake.

The finding of an endocytic property for liver parenchymal cells is unexpected. Further understanding of the cell surface behavior of these cells should be important not only in basic cell biology but also in the design of suitable carrier systems for introducing molecules of biological importance specifically into the liver parenchymal cells.

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1983

Lau, E. H., E. A. Cerny, B. J. Wright, and Y. E. Rahman
Improvement of Iron Removal from the Reticuloendothelial System by Liposome Encapsulation of N,N'-bis[2-hydroxybenzyl]-ethylenediamine-N,N'-diacetic Acid (HBED): Comparison with Desferrioxamine
J. Lab. Clin. Med. 101, 806-816 (1983)

MacCoss, M., J. J. Edwards, P. Lagocki, and Y.-E. Rahman
Phospholipid-Nucleoside Conjugates. 5. The Interaction of Selected 1-β-D-arabinofuranosylcytosine-5'-diphosphate-L-1,2-diacylglycerols with Serum Lipoproteins
Biochem. Biophys. Res. Commun. 116, 368-374 (1983)

Rahman, Y. E.
Liposomes as Carriers for Chelators
Liposome Letters, A. D. Bangham, ed., Academic Press, Inc., New York, NY, 1983, pp. 335-340

Rahman, Y. E., E. A. Cerny, E. H. Lau, and B. A. Carnes
Enhanced Iron Removal from Liver Parenchymal Cells in Experimental Iron Overload: Liposome Encapsulation of HBED and Phenobarbital Administration
Blood 62, 209-213 (1983)

Tom, B. H., C. M. Macek, L. Raphael, J. Sengupta, E. A. Cerny, M. M. Jonah, and Y. E. Rahman
Liposome Uptake into Human Colon Adenocarcinoma Cells in Monolayer, Spinner, and Trypsinized Cultures
Proc. Soc. Exp. Biol. Med. 172, 16-28 (1983)

1984 through June

Jonah, M. M., and Y.-E. Rahman
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, New York, NY, pp. 317-330

Schwendener, R. A., P. A. Lagocki, and Y. E. Rahman
The Effects of Charge and Size on the Interaction of Unilamellar Liposomes with Macrophages
Biochim. Biophys. Acta 772, 93-101 (1984)

Accepted as of June 30, 1984

Rahman, Y. E., K. R. Patel, E. A. Cerny, and M. MacCoss
The Treatment of Intravenously Implanted Lewis Carcinoma with the Sustained Release Forms of 1-β-D-Arabinofuranosylcytosine. A Covalent Phospholipid-Conjugate of ara-C and Liposome Encapsulated ara-C
Eur. J. Cancer Clin. Oncol.

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
C. A. Jones, J. P. Hardwick, K. Kiguchi, E. A. Malvoisin, S. Murao, C. T. Oravec, I. Simon, R. Grayson,* F. L. Lewis,† M. Stodolsky,§ M. F. Callaham, C. B. Henning, and B. A. Sedita

The objectives of this project are (1) to study, in cultured human and rodent cells, the mode of action of chemicals that initiate and/or promote tumor formation and (2) to establish simple and reliable cell culture systems for predicting the potential hazard of new chemicals that are being introduced into our environment.

A major question in current carcinogenesis research is why some carcinogenic chemicals exhibit cell or organ specificity. The mammalian cell-mediated mutagenesis assays that we have developed are useful for investigating some of the factors determining such specificity. In these assays, mutable target cells (e.g., Chinese hamster V79 cells), which cannot themselves metabolically activate chemical carcinogens, are cocultured with primary cells derived from a selected organ or tissue. Reactive intermediates generated from the carcinogenic chemicals by the primary metabolizing cells are transferred to the target cells where their mutagenic activity can be determined.

In one study, we used either fibroblasts or hepatocytes as the primary activating cells to metabolize benzo(a)pyrene (BP). We found that BP was activated to mutagenic intermediates by fibroblasts but not by hepatocytes. Analysis of the profiles of metabolites and DNA adducts obtained following incubation of hepatocytes and fibroblasts with [^{3}H]BP indicated that this cell specificity could be explained by a difference in the metabolism of BP in the two types of cells. In the hepatocytes, metabolism occurred principally in the K-region of the molecule resulting in the formation of BP-4,5-diol, phenols, and quinones, a major portion of which underwent subsequent metabolism to water-soluble conjugates. Only small amounts of BP-7,8-diol, the

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†Faculty Research Participant, Cheyney State College, Milwaukee.
§Visiting Scientist, Bosphorus University, Istanbul, Turkey.

precursor of BP-diol epoxide (BPDE), the ultimate carcinogenic and mutagenic metabolite of BP, were generated, and these were rapidly converted to nonmutagenic triols and tetraols. Metabolism of BP in the fibroblasts, on the other hand, was directed more toward the formation of BP-7,8-diol and hence BPDE.

These results indicate that the low susceptibility of the liver to BP-induced carcinogenesis may be explained by the preferential metabolism of BP via routes other than those leading to formation of significant amounts of BPDE. Thus, critical binding of this carcinogenic BP intermediate to hepatocyte DNA does not occur under normal circumstances.

Recently, we have isolated from a human teratoma cell line, a cell clone, designated P_3, which is useful for mutagenesis studies. These cells, which grow attached to the surface of Petri dishes, exhibit an epithelial cell morphology and have a stable diploid karyotype with 46(XX) chromosomes including a translocation between chromosomes 15 and 20. Resistance to 6-thioguanine, which is associated with a mutation at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus, can be induced in these cells. Analysis of a series of six P_3 mutants resistant to TG revealed that the acquisition of TG resistance in these cells was associated with a more than 10-fold reduction in HGPRT activity. These cells, however, in common with cells from other cell lines used in mutagenesis studies, cannot themselves metabolically activate chemical carcinogens. After cocultivation of the P_3 cells in a cell-mediated assay with human BJ carcinoma cells, which are capable of metabolizing polycyclic aromatic hydrocarbons (PAH), we could demonstrate that 7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, BP, and chrysene were capable of inducing TG-resistant mutants--with the degree of mutant induction related to the carcinogenic potency of the PAH. Pyrene and benzo(e)pyrene, which are not carcinogenic, were either inactive in this system or induced only limited TG resistance. Preliminary studies also indicated that P_3 cells can be used to detect the mutagenic response of dimethylnitrosamine in an assay when rat liver hepatocytes are used to activate the carcinogen.

These results thus indicate that the human epithelial teratoma P_3 cells can be useful in detecting mutations induced at the HGPRT locus by direct-acting mutagens or carcinogens and by premutagenic or precarcinogenic chemicals activated in a cell-mediated assay with either human or rodent cells.

Chemicals that have been shown to promote the formation of tumors in the mouse skin (such as the phorbol diesters and teleocidins), unlike complete carcinogens, do not bind to DNA and do not exhibit a mutagenic response. We and others have shown that the potent tumor promoters phorbol-12-myristate-13-acetate (PMA) and teleocidin B will induce the human HL-60 promyelocytic cell to differentiate into a

cell that phenotypically resembles a mature macrophage. These two chemically unrelated agents seem to exert their biological activity through a similar mechanism. Teleocidin competitively inhibits the specific binding of phorbol diesters to their cellular receptors and, in HL-60 cells, induces differentiation characteristics similar to those induced by PMA. Furthermore, an HL-60 cell variant (R-80) that we have isolated, which is resistant to PMA-induced cell differentiation, exhibits a similar resistance to teleocidin but not to other inducers of cell differentiation such as dimethylsulfoxide or retinoic acid.

Recently it was reported that 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], the biologically active metabolite of vitamin D_3, induced in HL-60 cells a number of differentiation markers. These studies raised the possibility that 1,25-$(OH)_2D_3$ may induce differentiation processes in the HL-60 cells via a mechanism similar to that of PMA and teleocidin. To examine this possibility, we compared the ability of 1,25-$(OH)_2D_3$ and PMA to induce cell differentiation in HL-60 cells and in two HL-60 cell variants that are resistant to PMA-induced cell differentiation. In addition, we investigated the ability of 1,25-$(OH)_2D_3$ to inhibit specific phorbol diester receptor binding as a means of establishing whether 1,25-$(OH)_2D_3$ shares similar binding sites with PMA and teleocidin.

The results showed that after treatment with 1,25-$(OH)_2D_3$, the HL-60 cells expressed a series of differentiation markers including an increase in lysozyme and nonspecific esterase activities, increased reactivity with OKM1 monoclonal antibody, and an increase in the fraction of morphologically mature cells. The induction of each of these markers was both time and dose dependent. The increases in nonspecific esterase activity and in the synthesis of a number of monocyte/macrophage protein markers and the absence of a large fraction of cells with banded or segmented nuclei (characteristic of granulocytes) indicate that, in contrast to previous suggestions, treatment of HL-60 cells with 1,25-$(OH)_2D_3$ produces cells that more closely resemble monocytes or macrophages than granulocytes. However the monocyte/macrophage phenotype induced by 1,25-$(OH)_2D_3$ in the HL-60 cells was similar, but not identical, to that induced by PMA.

We have also observed in these studies a resistance of our HL-60 cell variant (R-80) to the induction of cell differentiation by either PMA or 1,25-$(OH)_2D_3$. Together with the previous results, this resistance suggests that these two inducers may affect HL-60 cells by a similar process, which leads to the monocyte/macrophage-like phenotype. Even though these inducers do not seem to share common cellular receptors, it is possible that both receptors transmit similar signals to the cells, which results in comparable changes in the regulation of gene expression.

In summary, we anticipate that the work on the development and use of the various assays involving cultured human and rodent cells will lead to the development of rapid and reliable methods by which a broad spectrum of environmental contaminants can be tested for tumor initiating or promoting activity and organ specificity. In addition, this work should provide further insight into the cellular and biochemical events involved in the control of mutagenesis and cell differentiation and the development of neoplasia.

PUBLICATIONS APPEARING DURING THE NOTED PERIODS

1982 (received in 1983)

Ambrus, J. L., C. M. Ambrus, H. Gastpar, E. Huberman, R. Montagna, W. Biddle, S. Leong, and J. Horoszewicz
Antimetastatic and Antitumor Effect of Platelet Aggregation Inhibitors (ORNL work)
Interaction of Platelets and Tumor Cells, Alan R. Liss, Inc., New York, 1982, pp. 97-111

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Identifying and Estimating the Genetic Impact of Chemical Mutagens, N. Grossblatt, ed., National Academy Press, Washington, DC, 1982

Ryffel, B., C. B. Henning, and E. Huberman
Differentiation of Human T-Lymphoid Leukemia Cells into Cells that Have a Suppressor Phenotype is Induced by Phorbol 12-myristate 13-acetate
Proc. Natl. Acad. Sci. USA 79, 7336-7340 (1982)

1983

Au, W. W., M. F. Callaham, M. L. Workman, and E. Huberman
Double Minute Chromatin Bodies and Other Chromosome Alterations in Human Myeloid HL-60 Leukemia Cells Susceptible or Resistant to Induction of Differentiation by Phorbol-12-myristate-13-acetate
Cancer Res. 43, 5873-5878 (1983)

Jones, C., and E. Huberman
Studies on the Mode of Action of Chemical Carcinogens in Cultured Mammalian Cells (ORNL work)
Organ and Species Specificity in Chemical Carcinogenesis, R. Langenbach, S. Nesnow, and J. M. Rice, eds., Plenum Publishing Corporation, New York, NY, 1983, pp. 405-427

Jones, C. A., R. M. Santella, E. Huberman, J. K. Selkirk, and D. Grunberger
Cell Specific Activation of Benzo[a]pyrene by Fibroblasts and Hepatocytes
Carcinogenesis 4, 1351-1357 (1983)

4. RADIATION EFFECTS IN MAMMALIAN CELLS IN VITRO

C. K. Hill, Principal Investigator
A. Han,* M. M. Elkind,† R. L. Wells, E. M. Buess,
P. J. Dale, C.-M. Liu, J. M. Holland,§ and D. L. Redman,**

The purpose of this study is to explore the mechanisms for the radiation-induced changes in mammalian cells that lead to cell death, mutation, and neoplastic transformation. Of particular interest are the biology of low-dose-rate and dose-fractionation effects on these end points and 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 18 for further description of the radiation facilities of the Division.) To a lesser extent, we are also using nonionizing radiation.

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, and (3) labeling of DNA-precursor macromolecules with radioisotopes and measuring the damage to DNA with sedimentation and elution techniques. In further studies of neoplastic transformation in vitro, we test for altered properties by measuring the ability of cells to grow in semisolid agar and determine the tumorigenicity of transformed cells after their inoculation into suitable hosts.

Neoplastic transformation of mammalian cells exposed to fission neutrons or ^{60}Co gamma rays. During the past year, our studies of protracted exposures to fission

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neutrons (JANUS reactor) or ^{60}Co gamma rays have been continued and extended. We have now gathered enough data for ^{60}Co gamma rays to enable us to conclude that as for acute-dose-rate delivery, both low-dose-rate (0.1 rad/min) and multifractionated (50 rads/min) delivery produce transformation induction curves that are linear in the low dose region. However protracted exposures are significantly less effective than single acute exposures for both cell killing and the induction of neoplastic transformation. For neutron irradiation, we have strong evidence that the initial parts of the transformation induction curves for low-dose-rate and acute-dose-rate exposures are linear. In contrast to the results with ^{60}Co gamma rays, however, protracting the dose of neutrons by decreasing the dose rate to 0.086 rad/min significantly increases the induction of neoplastic transformation but has little or no effect on cell killing. Doses of neutrons delivered in several fractions at a high dose rate (10 rads/min) also enhance the transformation frequency. However, at high total doses where progressive disturbances (due to G2 blocking) occur in the cell cycle distribution, the transformation-frequency curve approaches the acute-dose-rate curve before it approaches that for equivalent low-dose-rate exposures. The initial slopes of the induction curves for the different exposure conditions with ^{60}Co gamma rays and fission neutrons have been used to estimate the relative biological effectiveness (RBE) of the different radiation qualities and doses by setting the dose of ^{60}Co gamma rays given at a dose rate of 100 rad/min as 1.00. These slopes indicate that the RBE may vary from 0.3 or less for multifractionated gamma ray exposures to more than 22 for low-dose-rate or fractionated fission neutron exposures. A study of a single dose (21 rad) of fission neutrons given at various dose rates indicates that the enhancement in neoplastic transformation begins to appear at dose rates below 0.43 rad/min and that the process involved can be completed in less than 50 minutes.

Mutation induction in mammalian cells exposed to ^{60}Co gamma rays and fission neutrons. In parallel with the transformation studies, an active study is under way 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. Preliminary data using 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 shape of the mutation induction curves are qualitatively similar. So far, with the dose rate reduced to 0.5 rad/min or 0.1 rad/min for fission neutrons, there appears to be no significant change in mutation frequency. The estimated RBE for induction of mutation by fis-

sion neutrons in the initial part of the induction curve is 15.

Role of promoters and inhibitors in the expression of radiation-induced neoplastic transformation. Studies using 0.1 μg/mL of tetradecanoylphorbol-13-acetate (TPA) added 24 h following irradiation and plating have shown that more than a 10-fold increase in transformation can occur in the small-to-moderate dose region at acute dose rates of ^{60}Co gamma rays or fission neutrons. When TPA is added after low-dose-rate exposures of ^{60}Co gamma rays, even though considerable repair has occurred, there is still a significant increase in transformation frequency, which suggests that some promotable damage either cannot be or is not repaired. Preliminary evidence with TPA added after irradiation with fission neutrons at low dose rates suggests that although the transformation frequency is already enhanced by the dose given at the low dose rate, there is still some increase due to TPA. However, the level of transformation reached is the same as that seen when TPA is added after single acute-dose-rate exposures, suggesting that some of the unexpressed damage that would normally be promoted by TPA has already been expressed. In some manner, then, it appears that low-dose-rate neutrons may facilitate the expression of the damage that they cause. In preliminary experiments with the protease inhibitor antipain, we found that the level of transformation after low-dose-rate exposures was reduced to the level seen after acute-dose-rate exposures. It remains to be seen how antipain affects acute-dose-rate exposures and thus what conclusions can be drawn about the mechanism of the enhanced transformation frequency at low dose rates of fission neutrons.

Mutation studies with monochromatic wavelengths of mid- to near-UV light. The studies with a sunlight-simulating source of near-UV reported last year have been extended by using single wavelengths of monochromatic light for irradiation. Using V79 Chinese hamster cells, we measured cell survival and mutation at the HGPRT locus and the sodium-potassium ATPase locus. As the wavelength increased from 313 nm to 405 nm, the frequency of induction of 6-TG resistant mutants decreased to a greater degree than did cell survival. No mutants resistant to either 6-thioguanine (HGPRT locus) or ouabain (sodium-potassium ATPase locus) were induced by 405 nm light and no mutants resistant to ouabain were induced by 365 nm light. Ouabain resistance was induced with considerably less efficiency at 313 nm and 334 nm than was 6-thioguanine resistance. These data indicate that the action spectra for the killing of cells and the induction of mutations are different from each other. In addition, action spectra for mutations at the HGPRT and Na/K ATPase loci are quantitatively and qualitatively different from each other. These observations imply major differences in the cellular mechanisms for cell inactivation, induction of 6-thioguanine resistance, and induction of ouabain resistance for V79 cells following

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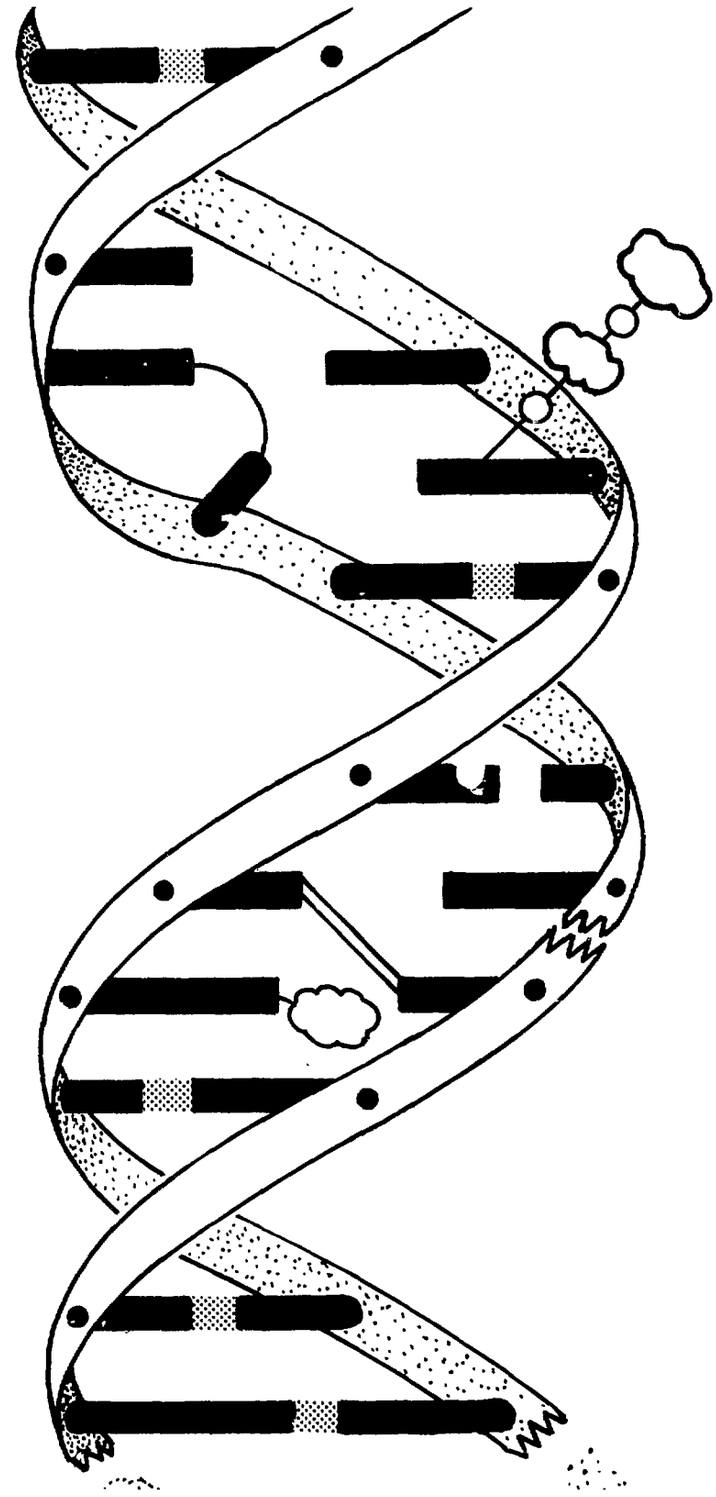

John J. Russell is cutting frozen sections of tissue on a cryostat. Tissue sections prepared in this manner are used in the studies described in Chapters 5-9, as well as in those described in Chapter 1.

5. 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 6 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 Chapter 6, "Life Shortening, Tumor Induction, and Tissue Dose for Fission Neutron and Gamma Ray-Irradiations"; in Chapter 4, "Radiation-Induced Changes in Mammalian Cells"; and in Chapter 1, "Mechanisms of Hepatocarcinogenesis."

Our studies of the mechanisms involved in radiation carcinogenesis include both *in vitro* and *in vivo* systems. Irradiations are performed 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 C3H10T1/2 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 (e.g., $LD_{50(6)}$ and $LD_{50(30)}$, respectively, following irradiation with and without protectors. The effect of radioprotectors on radiation- and/or

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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 experiments in progress are summarized as follows:

1. The effects of WR-1065 (4 mM) 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 cell killing by radiation and cis-DDP by factors of 1.7 and 1.8, respectively. Using resistance to 6-thioguanine in V-79 cells as an index of mutagenicity, we found WR-1065 to be effective in protecting against the mutagenic effects of both of these agents. In a representative experiment, the number of mutants per 10^6 survivors was reduced from 85 to 21 for 800 rad of radiation exposure and from 27 to 9 for 10 µg/mL of cis-DDP. The control mutation rate was about 7 per 10^6 survivors.

2. The ability of WR-1065 (1 mM) to protect against neoplastic transformation of C3H10T1/2 cells exposed to ^{60}Co gamma rays at a dose rate of 50 rad/minute was studied in collaboration with Dr. C. K. Hill (see Chapter 4). The transformation frequency per cell exposed was reduced at all radiation dose levels (e.g., 200 to 900 rad) when WR-1065 was present during irradiation. Maximum induction of transformation occurred at 500 rad (e.g., transformation frequencies of 1.3×10^{-3} and 3.5×10^{-4} for irradiation only and irradiation plus protector, respectively).

3. The agents WR-2721 and WR-151327 are being evaluated in collaboration with Dr. Peraino (see Chapter 1) with respect to their possible protective effects against the formation of enzymatically altered liver foci and hepatocarcinogenesis induced in newborn Sprague Dawley rats by ^{60}Co gamma rays, 0.85 MeV JANUS neutrons, and/or DEN. Preliminary studies suggest that WR-2721, administered at doses of 100 mg/kg 30 minutes before radiation or DEN exposure, can affect the production of these lesions. Studies in progress are assessing time-dose relationships.

4. WR-2721 and WR-151327 are being evaluated with respect to protection against acute radiation damage. C_3H mice irradiated with fission neutrons at doses ranging from 200 to 1100 rad were protected by WR-151327 from deaths related to intestinal factors [$LD_{50(6)}$] and deaths related to bone-marrow disturbances [$LD_{50(30)}$] by factors of 2.2 and 1.2, respectively. In contrast, WR-2721 exhibited protection factors of only 1.3 [$LD_{50(6)}$] and 1.2 [$LD_{50(30)}$], respectively. DNA damage from fission neutrons was evaluated with alkaline elution techniques. Preliminary data suggest that the protector WR-151327 may be effective in reducing DNA damage in both tissue systems, i.e., intestinal mucosa and hematopoietic cells.

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

D. Grahn, Principal Investigator
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F. S. Williamson, B. H. Farrington, G. L. Holmblad,
J. L. Hulesch, V. A. Ludeman, A. R. Sallese,
E. F. Staffeldt, J. E. Trier, and B. J. Wright

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 numerous specific types of tumors. This program is closely allied with that in Mammalian Genetics and Biostatistics (Chapter 7), in which genetic end points are examined using the same radiation sources, dose rates, total doses, and strain of mouse.

The animals are irradiated with fission neutrons from the JANUS reactor and ^{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.

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

Table 1. Exposure Types and Doses

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

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

The status of selected studies is as follows:

JM-9 extends the neutron dose-response curves to single doses below 20 rad and includes doses of 1, 2.5, 5, 10, 20, and 40 rad of neutron radiation and 22.5, 45, and 90 rad of gamma radiation. The results show that life shortening increases linearly with neutron dose up to 10 rad, at the rate of 5.6 days/rad. Gamma-ray-induced life shortening is 0.375 days/rad, which gives a limiting RBE of 15. Above 10 rad of neutrons, the response drops to ~ 2 days/rad.

JM-12 studies the effects of neutrons given in two, four, or six fractions at weekly intervals, compared with the same total dose (240 rad) given as a single exposure. Previous experiments had shown that life shortening after this dose was greater when the dose was divided among six, 24, or 72 fractions given over a 24-week period than that observed after a single exposure. The augmentation of neutron effect by dose fractionation is evident even with two fractions, and is most pronounced with four fractions. Analysis of causes of death shows that the augmentation is manifested differently for eac fractionation regime. After two fractions, there is a marked increase in early deaths from thymic lymphomas; after four fractions, both thymic and nonthymic lymphomas appear soon after exposure; and after six fractions, there is an accelerated development of lung tumors.

JM-4L involves gamma radiation only, delivered at instantaneous dose rates of 1.4, 2.8, 6.3, and 12.6 mrad per minute, 22 hours per day, five days per week, for a total of 23 weeks. The total doses accumulated are 206, 417, 959, and 1918 rad, the same as in a previous series of exposures in which the mice were irradiated once a week, to allow us to compare the effects of protracted gamma radiation at low dose rates with fractionated gamma radiation at high dose rates. The currently estimated life shortening coefficient is 0.16 days/rad accumulated, about 70% of that seen for once-weekly exposures.

JM-13, supported by the Nuclear Regulatory Commission, involves weekly exposures to neutrons (0.033, 0.125, 0.225, 0.35, 0.5, and 0.67 rad/weekly fraction) or to gamma rays (1.67, 3.33, 5, 7.5, and 10 rad/weekly fraction) for a total of 60 fractions (half of the life-span of the mouse). About 90% of the mice have died and provisional esti-

mates of the life shortening are ~ 0.2 days/rad of gamma-rays accumulated and ~ 3 to 4 days/rad of neutrons accumulated. Response is linear over the full gamma-ray dose range (100 to 600 rad) but only over the neutron dose range up to 21 rad.

Pathology

The gross autopsy and histopathology data have been divided into 26 diagnostic groups for both "cause-of-death" and "pathology present at death." Analyses of the pathological findings in all studies have begun to determine initially the net risk or probability of death from any tumor as a function of age, dose, sex, radiation quality, and exposure pattern. RBE (n/γ) values are 3 to 4 for all tumor deaths after single doses and 15 to 20 after 60 once-weekly exposures. This increase in the RBE values when exposures are fractionated is partly due to an increase in tumor risk when neutron irradiation is fractionated, but it is mostly attributable to the decrease in tumor risk that occurs when gamma irradiation is fractionated. Females have innately higher tumor risks per rad than males; however, this difference is not generally influenced by age or type of tumor, so that RBE values themselves are not significantly modified by sex. In general, epithelial tissue tumors show consistently higher RBEs than do connective tissue tumors. For example, after 60 once-weekly exposures, the epithelial tissue tumor RBE is between 40 and 50, while the RBE for connective tissue tumors is only 12 to 16. Tumor risk per rad increases exponentially between 400 and 800 days after exposure, then the risk tends to remain unchanged. This pattern is seen for both sexes and radiation qualities.

Dosimetry

All mice have been irradiated with each individual mouse caged in a 1-pint plastic cup. The estimated mid-line tissue dose has been based on phantom measurements and the assumption that the mouse rests in a prone position on the floor of the cup. $LD_{50/30}$ measurements for mice both in cups and in tubes, bilaterally exposed, are nearing completion and will be used to formulate a more realistic dose/kerma factor.

We are in the process of assembling a microdosimeter spectrometer with a 0.5-inch diameter proportional counter; this instrument will be used to document the distribution of events at representative locations in the neutron and gamma irradiation facilities.

The life shortening studies and analyses are almost completed; it is clear that the response to low doses of neutrons rises rapidly and linearly from 1 to 10 or 20 rad, regardless of the pattern of exposure, thus placing an upper limit on the RBE at low doses. This finding will be evaluated in terms of causes of death. Although we expect to find a wide variety of tumor-specific RBE values, we anticipate that the use of life shortening as a single integrated measure of lifetime injury will be substantiated and

eventually recommended as the basis for considerations of radiation safety. Specific tumor risks will not be ignored, however, as these provide a basis for more individualized conditions of protection.

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7. MAMMALIAN GENETICS AND BIOSTATISTICS

D. Grahn, Principal Investigator
B. A. Carnes, B. H. Farrington, C.-H. Lee,* and W. Finley†

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 18 for further descriptions of our radiation facilities). Breeding tests are performed with the irradiated males at selected periods to obtain data on postmeiotic, meiotic, and premeiotic cell stages in male gametogenesis.

Effects from single neutron doses of 1, 2.5, 5, 10, 20, and 40 rad have been compared with the effects of 22.5, 45, and 145 rad of ^{60}Co gamma rays; a weekly exposure series has also been performed for additional neutron and gamma ray comparisons at low weekly increments of 0.67, 1.67, and 2.67 neutron rad vs. 6.95, 17.4, and 32.0 gamma-ray rad 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 (see Chapter 6 for a summary of the somatic effects). The genetic tests evaluate the

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†Summer 1983 Student Research Participant, Lehigh University, Bethlehem, PA.

effects of 0.125, 0.35, and 0.67 rad/week of neutrons and 5, 7.5, and 10 rad/ 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.

Analysis of the genetic responses. In the single dose studies, we find a continuous linear response for the induction of dominant lethal mutations in postspermatogonial stages for exposures down to 5 rad. Below this level, there is a significant response in excess of linear prediction, which is about a factor of two above expectation. This excess level of response is noted in the rates of both postimplantation and preimplantation losses. Altogether, significant genetic damage is detected for all end points at 2.5 rad, and for all except abnormal sperm at 1 rad. In studies involving once weekly irradiations, where males are mated periodically during the 6-day radiation-free period, statistical reduction of the internal error becomes critical for the evaluation of dose-response relationships because responses are near background. The heterogeneity of mutation rates among litter sizes within doses was examined as a potential basis for reducing the variance. Stable mutation rates were observed for litters with between 6 and 11 pups, so a statistical filter of the data was established. Comparisons of variance analyses with and without the filter indicated that basic response patterns were not altered, but the significance levels of differences in response were increased by the reduction of the internal error in the filtered data.

The filtered data were then used for the analyses of dose-response data for both preimplantation and postimplantation losses. The regressions of response on dose were constrained through a common intercept (the control) to facilitate comparisons between gamma and neutron irradiated animals. Preimplantation loss could not be adequately described as a simple function of dose (R^2 = .07); however, the data on postimplantation loss (mutation rate) were adequately modeled (R^2 ~ .98 for both radiation qualities) by a simple linear dose-response equation. The slope or mutation rate for neutrons (.0433 ± .00365/gamete/rad/week) was 11-fold larger than the slope for the gamma-ray data (.0040 ± .00039/ gamete/rad/week).

To clarify the data on the question of whether augmentation of injury by protraction of the neutron dose has occurred, we performed a multiple regression analysis on the mutation rate data using a second independent variable--week of mating--which is a surrogate for accumulating stem cell dose/damage. This analysis accounts for time-dependent variation in the mutation rate that is independent of variation specifically related to dose. As before, the dose - dependent increase in mutation rate/gamete/rad/week was significantly larger for neutron-irradiated animals (.04421 ± .00338) than for gamma-ray-irrad-

iated animals (.00370 ± .00078). The increase in mutation rate/week was similar for both neutrons (.00068 ± .00023) and gamma rays (.00078 ± .00024). If these coefficients are divided by the mean dose/week, they become estimates of the mutation rate/gamete/rad of accumulated dose to the spermatogonia. The two estimates are 5.4 x 10^{-4} (neutrons) and 5.5 x 10^{-5} (gamma rays). Direct estimates from preliminary analyses of mutation rate data are in the range of 2.7 to 4.4 x 10^{-4} for neutrons and 2.8 to 3.5 x 10^{-5} for gamma rays. The differences between the two sets of estimates are not significant, but analysis of the data on dominant lethal mutations induced in the stem cell population needs to be refined. One can conclude from the multiple regression analysis that significant augmentation of genetic injury is induced by neutron irradiation protracted by low dose rate delivery. The rate of 0.0442/gamete/rad/week, when divided by five (the number of weeks between the last spermatogonial stage and the mature sperm), is 0.0088, which is significantly greater than the rate of 0.0052 ± 0.0001 seen when males are mated for a full 5 weeks following a single high-dose-rate exposure to fission neutrons.

The collation of genetic data with the data on long-term somatic effects reveals that the RBEs for genetic effects are generally less than those for long-term somatic effects, probably because the latter reflect multisystemic pathological interactions that amplify damage. Genetic injury is not subject to as much complication by systemic interactions and can also be categorized by specific cell-stage factors. Meiotic and postmeiotic stages, for example, show a narrower spread and lower limit to the RBEs because of the limited opportunities for the repair of genetic damage. The augmentation of neutron-induced injury by dose protraction is a common finding for in vivo measures of cellular and systemic injury; this augmentation is never more than a factor of two, which is significantly less than that seen in the in vitro studies reported in Chapter 4.

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8. RADIATION TOXICITY STUDIES IN DOGS

T. E. Fritz, Principal Investigator
L. S. Lombard, C. M. Poole, T. M. Seed, D. V. Tolle,
S. M. Cullen, D. E. Doyle, G. L. Holmblad, L. V. Kaspar,
W. G. Keenan, A. R. Sallese, and M. W. Elliott*

The basic objective of this program is to generate comprehensive data on the late effects of low doses of ionizing radiation in a large, relatively long-lived animal, the dog--information necessary to provide a basis for extrapolations of data from laboratory animals to man. The specific objectives are (1) to determine the influence of two major factors, the daily exposure rate and the total accumulated dose, (2) to provide data for estimates of radiation-specific excess mortality in the dog to enable interspecies comparisons to existing rodent data and to develop a unifying concept of radiation damage, and (3) to 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/day, 7 days/week at various exposure rates down to those allowing a nearly normal life span. Radiation is given either until death of the animal (0.4, 1.0, and 2.5 R/day) or until predetermined total doses (600, 1400, 2000, and 4000 R) are accumulated. The total doses are delivered at exposure rates of 5, 10, 17, and 35 R/day to allow us to compare results with those from dogs previously irradiated until death at these exposure rates. The dogs are maintained for their entire life span and are monitored and evaluated at regular intervals by hematologic, clinical, and pathological examinations, and a complete necropsy is performed at death. All data are entered into a computerized data base.

Responses of adult beagles to continuous exposure to ^{60}Co gamma rays. We have continued to monitor both control dogs and those under continuous irradiation at 2.5, 1.0, and 0.4 R/day. As of May 1984, these dogs had been irradiated for times ranging from 1934 to 3004 days after their entry into the experiment at 400 days of age. All dogs are in generally good health; the irradiated

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dogs cannot be differentiated clinically from the controls, except for depression in the numbers of circulating blood cell and testicular atrophy in males.

The deaths since last year of an additional 7 dogs gives a total of 27 decedents (23 experimental dogs out of a total of 184 and 4 controls out of 46). Almost 25% of the dogs in the 2.5 R/day group have died, but only 12% in the 1.0 R/day group and 5% in the 0.4 R/day group, respectively, are dead. In the latter two groups, only one of the deaths is believed related to the irradiation. This death was from myelofibrosis and terminal anemia in a dog irradiated at 1.0 R/day and dying at 1829 days of irradiation. In the 2.5 R/day group, 10 of the 13 deaths were from causes related to the hematopoietic system: 7 were from myelogenous leukemia or related myeloproliferative disorders (MPD), 1 was due to aplastic anemia, and 2 were caused by mast cell tumors. A third mast cell tumor occurred in one of the dogs dying of leukemia. Three cases of mast cell tumors among the 13 decedents suggests that mast cell tumors may be a significant finding.

As reported previously, we found early depression of thrombocyte and leukocyte values and exposure-rate-dependent differences in the peripheral blood values for dogs from the three groups. In earlier reports, we pointed out that these differences are not seen when the blood values are plotted against total accumulated exposure rather than against time in the radiation field. For the dogs undergoing continuing irradiation, the values for both thrombocytes and leukocytes are stabilizing at different exposure-rate-dependent levels when the data are plotted against total dose.

In earlier studies at dose rates above 5 R/day, we showed a relationship between radiation injury (as measured by increased radiation mortality) and dose rate in the dog that is parallel to that of the mouse. On a log-log plot the relationship conforms to a slope 2 response (i.e., linear with the dose squared); therefore, the response is determined by the dose rate. These current studies will permit us to determine the relationship of total dose and dose rate to biological response at lower dose rates and establish a unifying concept of radiation damage that is useful in interspecies extrapolations. Estimates of the mean aftersurvival times (MAS) for the dogs at the three lower exposure rates suggest that the MAS established values conform to a slope of 1. In such a case the dog will show a sensitivity similar to that of the mouse, the response will be independent of dose rate, and the injury will be additive and determined by total dose as in the mouse.

Responses of adult beagles to terminated exposures to ^{60}Co gamma rays. The dogs still alive in this study as of May 1, 1984, range in age from 2343 to 5152 days. Forty-seven percent of the irradiated and 52% of the control dogs have died. Although the data

are incomplete, we can base several tentative conclusions on the interim observations and results to date:

1. The total incidence of fatal tumors is at present greater in the irradiated group than in the control group ($P = .03$).

2. As identified in earlier reports, there continues to be significant life shortening as measured by survival times in the combined irradiated groups as compared to controls. For all causes of deaths, the MAS is now 2627 ± 87 days (SE) for the irradiated dogs compared to 3521 ± 201 days (SE) for the controls ($P < .001$). For dogs with fatal tumors, the difference in MAS is even greater [2757 ± 110 days for irradiated and of 3883 ± 198 days for controls ($P < .00001$)].

3. Irradiation does not appear to influence the relationship between carcinomas and sarcomas (53% of the tumors in the irradiated dogs were carcinomas vs. 57% in the controls).

4. Both sarcomas and carcinomas are occurring significantly earlier in the irradiated dogs than in the controls. The time to death from the two different types of tumors is not significantly different in controls, but in irradiated dogs sarcomas occur much earlier than do carcinomas. Thus, the greater proportion of the life shortening in irradiated dogs is due to the greatly accelerated induction of sarcomas.

5. A majority of the life shortening in the irradiated dogs can be ascribed to four types of sarcomas: lymphosarcomas, lymphocytic leukemias, myelogenous leukemias, and splenic fibrosarcomas.

6. Malignancies of the mammary gland also occur much earlier in the irradiated dogs than in the controls, although the incidence is not greater at present.

7. On the basis of the survival data from four groups in which all or nearly all dogs are now dead, we can now tentatively estimate a coefficient of life shortening for interspecies comparisons. The calculated or estimated median (50%) survival time from plots of cumulative aftersurvival were used to derive the MAS.

On the basis of the MAS, we calculated the life shortening in days per R of irradiation (d/R) according to the following:

$$\frac{MAS_0 - MAS_I}{E} = d/R$$

and the percentage of life shortening per 100 R (% LS/100 R) according to the following:

$$\frac{d/R \times 100}{MAS_0} = \%\ LS/100\ R$$

where MAS_0 = mean aftersurvival of control animals, MAS_I = mean aftersurvival of irradiated animals, and E = total radiation exposure in Roentgens.

The mean percentage of life shortening per 100 R for dogs given terminated exposures of gamma rays from a ^{60}Co source is 1.35 ±

.32. This value is similar to that for life shortening resulting from protracted irradiation of the mouse, 1.7% ± .18/100, a value that was determined with total doses in the range of 200-2000 rad protracted over 24 weeks (see Chapter 6 of this report).

Thus, these interim estimates for both continuous and terminated exposures suggest that despite large differences in sensitivity to acute brief doses of irradiation, body size, and life span between the dog and mouse, there may be a similar biological value for risk of life shortening and radiation damage for the two species.

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In conclusion, the radioprotectors evaluated thus far have been shown to protect against radiation-induced DNA damage, cell killing, mutagenesis, and transformation. As such, they offer the potential of being a class of agents that can 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 fission neutron as compared to low LET radiations.

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Workshop on Responses of Different Species to High Dose Total Body Irradiation; Rijswijk, The Netherlands; July 1, 1983

9. HEMATOPOIESIS IN CHRONIC TOXICITY STUDIES

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

Aplastic anemia and myelogenous leukemia are, under select conditions, prominent pathological results of exposure to ionizing radiation. To establish "estimates-of-risk" for both single and protracted radiation exposures, we must consider these hematological pathologies--not only in terms of their incidence under various exposure regimens but also in terms of the radiation-dependent processes involved in the induction and progression of these syndromes within "high-risk" individuals. The primary objective of this project, therefore, is to elucidate the mechanisms by which chronic ionizing gamma irradiation induces and promotes the development of aplastic anemia and myelogenous leukemia.

Our approach has been to assess sequentially the changes in blood-forming tissues of beagles chronically gamma irradiated with ^{60}Co gamma rays (2.5-17 R/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 being applied to assess the structural changes in hematopoietic tissue during preclinical stages. Cell cloning assays are being used to sequentially monitor vital progenitor populations of both hematopoietic and stromal cell origin. The care and clinical monitoring of the animals included in this work is supported by the staff of the Radiation Toxicology Group* and radiological assistance is provided by a member of the Radiation Biology Group†.

During 1983, we continued to elucidate the cellular mechanisms underlying hematopoietic recovery in beagles under chronic gamma irradiation. This recovery appears to be a pivotal process in the induction and progression of both aplastic anemia and MPD. Major areas of effort include (1) structural studies of the sequential changes in the architecture of the bone marrow during the prerecovery and early and late postrecovery periods and (2) functional studies of the sequentially

*Radiation Toxicology Group: D. V. Tolle and S. M. Cullen, hematology; D. E. Doyle, data processing; T. E. Fritz and L. Lombard, pathology; and C. M. Poole and W. G. Keenan, veterinary care.
†G. L. Holmblad.

collected, marrow-isolated hematopoietic progenitors, relative to radiosensitivity, cell cycle status, and repair capacities.

Structural studies. Ultrastructural analyses of marrow specimens from aplasia-prone or MPD-prone dogs, which were taken during the prerecovery period (50-150 days of exposure), revealed marked differences in the endosteum (i.e., the functional critical cellular interface between bone and marrow). In the marrow specimens of the MPD-prone animals, the endosteum exhibited markedly increased cellular activity; focal endosteal areas were transformed from predominantly quiescent to formative states. This activity is in contrast to the increased quiescence (decreased formative areas) and focal degenerative lesions of the marrow endosteum of the aplasia-prone dogs during this early exposure period. The increased endosteal activity of the MPD-prone dogs coincided with the initial restructuring of the marrow's reticular and vascular network (ie., the hematopoietic microenvironment) and preceded the subsequent regenerative (recovery) hematopoietic response. These endosteal changes appear to be early "repair" processes, essential for hematopoietic regeneration, long-term survival, and the development of MPD.

Functional studies. We have confirmed and extended our observations concerning the radiation dose- and pathology-dependent acquired shifts in radiosensitivity of hematopoietic progenitors (CFUa-GM) of dogs under chronic gamma irradiation. Our results indicated that the radiosensitivity of CFUa-GM from dogs prone to aplastic anemia, irradiated at either 17 or 10 R/day, was not altered appreciably during the course of exposure and pathological progression. In contrast, the CFUa-GM from MPD-prone dogs, irradiated at either 5 or 10 R/day, showed increased radioresistance during the hematopoietic recovery phase (phase II) following the initial period of acute hematopoietic suppression (phase I). Radioresistance of CFUa-GM was maintained during the subsequent accommodation phase (phase III) and continued into the late preclinical phase of developing MPD (phase IV). Acquired radioresistance was characterized by three distinct patterns of response: A, elevated high-dose tolerance; B, elevated low-dose tolerance; and C, combined, elevated high- and low-dose tolerance. The frequency of these responses varied during preclinical MPD phases II-IV. Variants A and B declined from 67% and 33% to 46% and 9%, respectively, while variant C increased from 0% to 36%. These changes reflect a time-dependent evolution of specific clonal subpopulations of hematopoietic progenitors during the preclinical progression of MPD.

During this year we have continued to elucidate the cellular/molecular mechanism(s) underlying acquired radioresistance by vital hematopoietic progenitors of MPD-prone dogs. Selective changes in cellular repair capacity, cell-cycle properties, and microenvironmental effects, have been con-

sidered and partially assessed. The change in recoverability (survival ratios) following dose fractionation and the ablative effects of high LET neutron irradiation on the capacity to accumulate sublethal damage suggest that modified repair does occur. Phase-specific cell-cycle modifications have been indicated by toxicity assays (i.e., S-phase-specific ARA-C suiciding assays) indicating enhanced cell cycling prior to hematopoietic recovery and suppressed cell cycling during the post-recovery phases. The potential microenvironmental effect of chronic tissue hypoxia has been ruled out by work with O_2-dependent radiosensitizers.

In conclusion, the identification and partial characterization of specific preclinical phases clearly indicates the "multistaged" nature of chronic radiation-induced myeloproliferative disease. Further, aberrant hematopoietic recovery (repair) appears to be a pivotal stage, mediated, in part, by an acquired change in radiation sensitivity of targeted hematopoietic stem cells.

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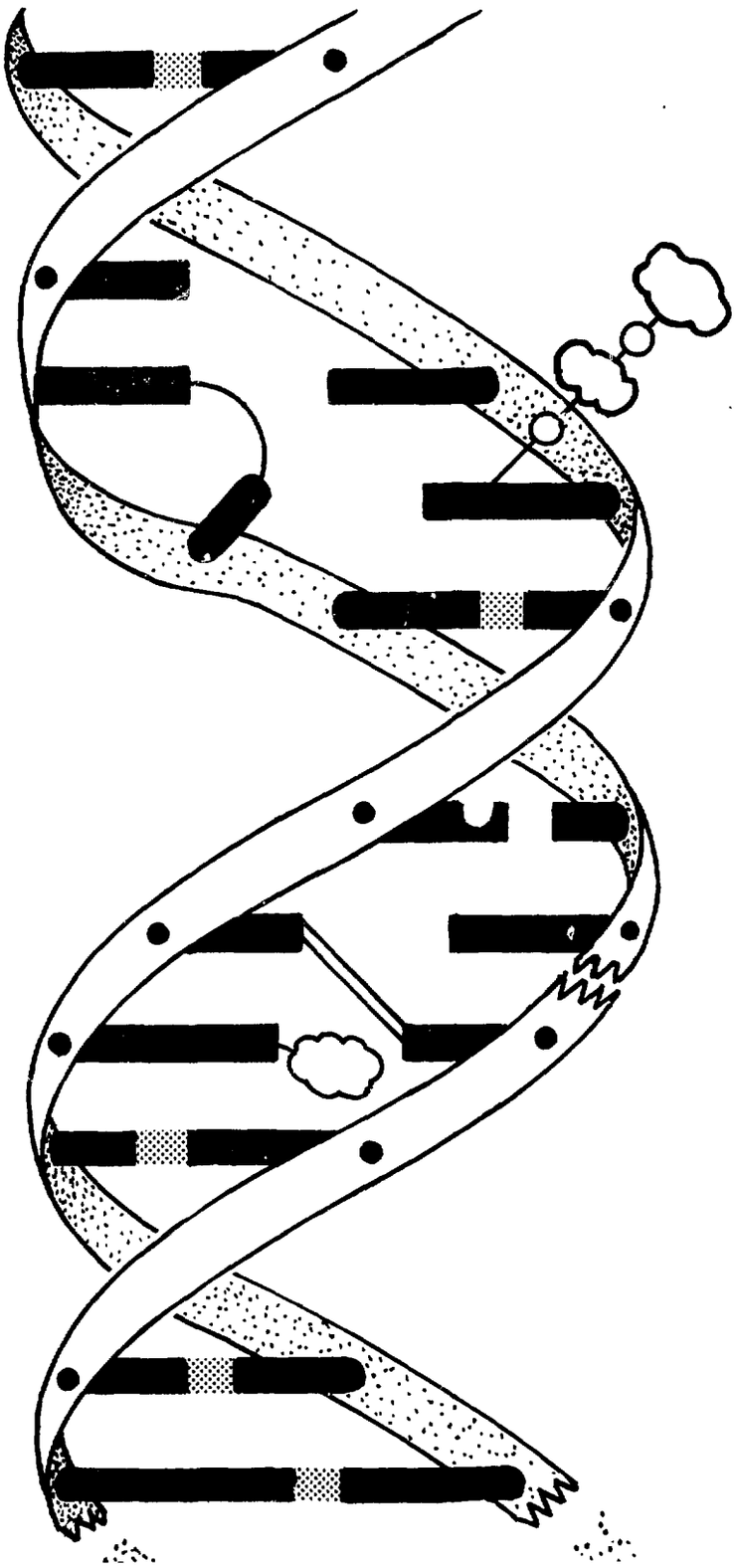
Molecular Biology



Dr. Jennifer G. Peak is harvesting human cells that have been irradiated with ultraviolet radiation for analyses of damage to DNA. See Chapter 10 for a description of the research program involved.

10. MOLECULAR AND CELLULAR EFFECTS OF RADIATIONS

M. J. Peak, Principal Investigator
J. G. Peak, M. P. Moehring,* M. A. Turner,†
R. M. Jacobs,§ and R. S. Sikorski**

This program is concerned with the basic nature of the biological effects of mutagenic and carcinogenic environmental radiations, especially those solar ultraviolet and visible radiations responsible for the commonest form of human cancer, cancer of the skin. The program concentrates upon the damages to DNA caused by these radiations and attempts to delineate the basic mechanisms whereby such changes in the genetic material 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. Biological systems used as targets range from procaryotic cells and subcellular genetically active nucleic acid to eucaryotic cells in culture such as 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), alkali labile sites, DNA-to-protein covalent bond crosslinking, and the formation of DNA photoproducts such as pyrimidine dimers and pyrimidine adducts. This work has identified two principal types of pathways through which damage 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

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**Summer 1983 Student Research Participant, Pennsylvania State University.

are studying the details of these various mechanisms and the ultimate damages that they inflict upon DNA, damages that may produce the genetic changes that result in carcinogenesis. The following summarizes some of our recent results.

DNA-to-protein covalent bond formation. We have extended our observation that DNA-to-protein covalent bond crosslinking is produced in Chinese hamster V79 cells by monochromatic 365 nm and 405 nm radiations by exposing both V79 and human cells to other regions of the electromagnetic spectrum. An alkaline elution method was developed, which improves the quantification of the yield of this lesion such that linear dose response kinetics can be obtained, not possible with the proteinase k method we used previously. In our new method the cells are irradiated with the monochromatic wavelengths, then further treated with x-rays or gamma rays to break the DNA into small fragments. The proportion of these fragments retained on the filter after alkaline elution is then measured. Dose responses for the induction of DNA-protein crosslinks in human P_3 cells by radiation at 254, 290, 313, 334, 365, 405, 434, and 545 nm were determined. All these radiations induced measurable crosslinks, except for the green light at 545 nm. Estimates of the number of crosslinks/genome/lethal event at the various wavelengths showed that the near-UV and blue light exposures induced about three orders of magnitude more of these lesions than did the more energetic far-UV radiation. This finding is significant because it is these longer wavelengths that penetrate the skin and irradiate the dermis and peripheral blood cells. For instance, radiation at 405 nm causes more than 2,000 crosslinks / cell genome / lethal event. At this wavelength, about 20% of the energy incident on the surface of the skin reaches the dermis. A spectrum delineating the relative efficiencies for the formation of DNA-protein crosslinks was prepared. This spectrum reveals that the crosslinks are induced by at least two mechanisms that depend upon the energy of the radiation. In the high-energy region (254-313 nm), the close correlation between the crosslink yields and the absorption of DNA indicates that the crosslinking occurs as a result of the direct absorption of photons due to the tautomeric resonance of the nucleic acid bases. At the lower energy region of the crosslink-inducing radiation, no such correlation exists between the crosslink yields and the absorption of DNA, indicating that a nonDNA intermediate is perhaps the primary chromophore. This lack of correlation manifests itself as a minor peak, λ_{max} at about 405 nm, close to the absorption maximum for porphyrin residues. The fact that there is no oxygen requirement for the induction of crosslinks by 290 nm radiation, whereas at least 85% of the crosslinks caused by 405 nm radiation require the presence of oxygen, supports the conclusion that in the more-energetic region damage is produced by direct action whereas in the less-energetic

region an indirect photodynamic reaction occurs. These observations support the possibility of more than one type of crosslink.

DNA pyrimidine photoproducts. Pyrimidine adducts (6-4'-pyrimidine-2'-one-pyrimidine) have recently been pinpointed as a new radiation-induced DNA lesion that has importance in mutagenesis. Action spectra for the induction of pyrimidine dimers (of the 5-6 cyclobutane type) and pyrimidine adducts were prepared by use of DNA sequencing techniques that detect the lesion-specific sites in the DNA. This technique involved a 127 base pair DNA fragment of defined sequence (studied in collaboration with Dr. W. A. Haseltine, Harvard Medical School). This action spectrum is the first prepared for pyrimidine adducts and the first for both photoproducts using site-specific analysis. These spectra were compared with spectra for mutagenesis, dimer induction measured by classical chromatographic techniques, and the absorption of DNA. All these spectra correlated in the spectral region of high energy (below 320 nm), indicating that the adduct, as well as the dimer (which is well known to be mutagenic), may indeed be another mutagenic lesion. At lower energies, the situation is less clear. At 334 nm, for instance, all measurements (dimers measured by chromatography or by DNA sequencing and adducts measured by sequencing) showed more photoproducts than we would expect to be produced by DNA absorption. This evidence is the first that at 334 nm, pyrimidine dimers and pyrimidine adducts may be induced by both indirect and direct mechanisms.

DNA backbone scissions. Currently a controversy exists as to the biological role of DNA backbone scissions caused by nonionizing and ionizing radiations. Comparisons of action spectra for the killing of different species and strains of procaryotic cells with spectra for DNA backbone scission shows that the species and strains that are killed most easily are also the most sensitive to DNA scissions. This observation suggests that these breaks may cause cells to die. Use of isolated genetically active DNA has provided further evidence that DNA breaks play a role in DNA inactivation. An action spectrum for protection by glycerol against the inactivation of the genetic activity of the nucleic acid was compared with a spectrum for protection against the induction of breaks. These spectra closely matched each other, thus providing evidence for a role of DNA backbone scissions in the inactivation of the genetic activity of DNA. However, we have not excluded the possibility that some other lesion imposed by the radiation in the same ratio as it imposes backbone breaks may be the critical lesion.

Photodynamic action. In collaboration with Drs. C. S. Foote and N. I. Krinsky, Chemistry Department, UCLA, and Department of Biochemistry and Pharmacology, Tufts University, respectively, 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. New evidence for a role of singlet oxygen is that irradiation of DNA by 334 nm in an environment of D_2O (compared with H_2O) markedly enhanced the induction of breaks in the DNA. New evidence for a role for superoxide anion is that superoxide dismutase protects DNA by a factor of 0.2 from breakage caused by 334 nm radiation.

It has not yet been demonstrated whether singlet oxygen has the excitation energy required to break DNA. We used rose bengal plus green light, a system considered to be specific for the production of singlet oxygen, to generate singlet oxygen in the presence of DNA of known molecular weight. We showed that this system induces breaks in DNA; however, control experiments (irradiation in the presence of D_2O compared with H_2O, irradiation in the presence of diazabicyclo-[2.2.2.]octane, irradiation in the absence of oxygen) revealed that rose bengal plus green light can react directly with DNA, without the intervention of singlet oxygen. This observation casts doubt upon all previous work investigating the biological effects of singlet oxygen where rose bengal was used to generate this reactive species.

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11. THE MOLECULAR ANATOMY PROGRAM

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

The primary focus of the Molecular Anatomy Program is the description of cellular processes and cellular injury at the molecular level. In this effort, two approaches can be used: (1) We can examine a very few molecular species in cells and assume that these few are representative, or (2) we can develop global analytical methods that allow a large number of different species of molecules to be seen. We have chosen the latter approach, which has required the development of new systems and techniques for separation and analysis of various cellular constituents. Our initial efforts included the development of high-resolution chromatographic systems for the separation and analysis of low-molecular-weight constitutents of cells, the development of zonal centrifuges for subcellular fractionation, and the development of centrifugal fast analyzers for rapid and precise analyses based on enzyme activity or immunological specificity.

The major function of cellular DNA is to code for proteins, which carry on nearly all cellular functions including the synthesis and repair of DNA itself. Proteins thus reflect alterations in DNA, are in themselves the end result and best description of differentiation, and also, through changes in types and amounts synthesized, reflect the effects of pollutants, carcinogens, toxic agents, drugs, and hormones. Our present approach, therefore, focuses on the separation and analysis of proteins by two-dimensional (2-D) electrophoresis.

The number of proteins coded for by one human genome is not known, but estimates range from 30,000 to as many as 100,000, well beyond the analytical range of previously available techniques. The technique, high-resolution 2-D electrophoresis, offers the possibility of resolving the very large numbers of proteins found in human cells. For maximum usefulness, the system should have high-resolution, allow large numbers of reproducible analyses, identify as many proteins on the 2-D maps as possible, and include image analysis and data reduction systems

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that will allow all of the protein spots to be resolved and will also allow large numbers of images to be intercompared. The ISO-DALT electrophoretic system developed at Argonne enables more than 10,000 analyses to be completed per year. Reference maps of human serum proteins, muscle, fibroblasts, lymphocytes, red cells, platelets, urinary proteins, and seminal plasma have been prepared and published. These maps are serving as reference standards, which allow disease-associated changes to be detected.

Of continuing interest in our program has been the improvement of resolution. The theoretical resolution obtainable is now more than 30,000 proteins, and we do not believe that the limits of resolution have been reached. Hence, we are investigating the use of larger gels and immobilized pH gradients.

Large integrated computer programs for image analysis and data reduction have been written (the TYCHO and KEPLER Programs); these programs are now considered the standards for this field and are being shared with other laboratories.

We have examined in detail the problem of using high-resolution electrophoresis for detection of mutation in mammalian cells. More than 70% of base substitutions (point mutations) in the DNA of a structural gene result in an amino acid substitution in a completed protein, and one-third of such substitutions result in the addition or deletion of a charge on that protein. The addition or substraction of a single charge from a protein is easily detected on 2-D gels over the molecular mass range of approximately 10,000 to 150,000 daltons. One protein containing 400 amino acids therefore serves to monitor approximately 300 bases. If 1000 proteins can be examined on a single electrophoretic gel, then the equivalent of one-third of a megabase of DNA can be examined for point mutations in one analysis. This method is the most promising wide-range mutation detection method currently available and has strong potential for the detection of both radiation- and chemically induced mutations and for the measurement of mutation rates.

These systems have been applied to the exploration of the tropomyosin family of proteins in human cells; several new genetic variants of tropomyosin have been discovered, demonstrating that new genetic variants can be readily found. For each new variant found, however, it is necessary to prove that the variant is indeed a variant of an identified wild-type protein and not a different, adventitious, or unrelated protein that has accidentally appeared next to a normal cell protein. In model studies using tropomyosin variants, we have developed methods for relating a variant to its wild-type protein, including the demonstration that the variant and wild type co-isolate during biochemical isolation, that their partial proteolytic digests give identical peptide maps, that both react with antibodies against the wild-type protein on nitrocellu-

lose transfers, and that they have similar amino acid compositions. These techniques may now be applied systematically to other new variants as they are discovered.

The discovery of genetic variants and the demonstration of their relationship to a wild-type protein are now straightforward, as are the methods of measuring mutation rates. However the problem of how to detect, measure, and understand changes in gene expression (and hence changes in the rate of synthesis of individual proteins and in their steady-state amounts) after exposure of cells to drugs, toxic pollutants, carcinogens, or hormones is more difficult to solve because the effects may involve many proteins.

Initially seventeen different treatments or agents including some antibiotics, heat shock, phorbol esters, interferon, and cadmium were examined for their effect on cells in culture, and the cellular proteins affected in a major way by each were indentified. These treatments, however, cause many proteins to be affected to at least a small degree, and the time courses of these effects were often different for different proteins. Hence, we are now developing more sophisticated multifactorial statistical analytical methods to allow these effects on cellular proteins to be described in greater detail and to be intercompared.

The production of energy from biomass has resulted in interest in the development of improved plant energy sources. We are now exploring the application of high-resolution 2-D electrophoresis to the description of plant phenotypes and to the search for genetically variant proteins or protein groups that are associated with increased yields. The initial problem has been to adapt our analytical systems to well-studied plant proteins including wheat endosperm proteins. Using known wheat varieties, we have been able to discover new variants (biotypes) and to demonstrate relatedness among variants using the techniques we developed for human cells.

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12. STRUCTURAL STUDIES OF PROTEINS

M. Schiffer, Principal Investigator
C. S. Borso, F. J. Stevens, E. M. Westbrook, C. H. Chang, M. T. Short, P. Thiyangarajan, C. W. Hanly,* R. M. Hyslop,† C. F. Ainsworth, F. A. Westholm, R. McMasters,§ and B. Shen**

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 structural 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, and calmodulin. Information is obtained by various biophysical techniques including x-ray diffraction for crystalline material and small angle x-ray scattering and gel filtration for molecules in solution.

Immunoglobulins. Immunoglobulin light chains are excellent models for intact antibody molecules. We are attempting to understand how antibodies function and explain their high degree of specificity through structural studies conducted by x-ray diffraction of single crystals of immunoglobulin light chains (Bence-Jones proteins) excreted by patients with multiple myeloma. To extend the structural information we originally obtained from the λ_V-type Bence-Jones protein Mcg, we have purified, characterized, and attempted to crystallize more than 50 Bence-Jones proteins from different light chain classes and subgroups; of these, we have crystallized and started structural determination on four.

During the past year, the structure of λ_I-type Bence-Jones dimer Loc was determined and refined at 3 Å resolution; the crystallographic R factor is 27%. The antigen binding site formed by the two variable domains of the Loc light chain dimer differs significantly from that of any antigen binding fragment (Fab) or light chain studied thus far.

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This difference in the conformation of the binding site is caused not only by changes in the hypervariable regions, which no longer form a cavity, but also by differences in the relative positions and interactions of the variable domains. The structure of protein Loc indicates that immunoglobulins are capable of forming a much more diverse spectrum of antigen binding sites than those observed up till now; the variability of the antigen binding sites may be significantly enhanced by changes in the relative positions of the variable domains.

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 auto-antibodies, are antibodies against the most common antibody molecule, IgG, and have been observed in a number of clincial conditions. To understand the function of these antibodies in terms of their three-dimensional structure, we are studying their binding properties in solution and are attempting to crystallize them for structural determinations.

During this year, we developed the procedure for purification of the IgA rheumatoid factor Sch from plasma and prepared the Fab fragment from it with IgA protease. Chromatographic methods were used to estimate the binding constant of the Fab fragment with the Fc fragment from IgG1 protein Dri. The binding constant is $\sim 10^5$ M^{-1}, a value similar to the ones observed for other rheumatoid factors. This binding strength is high enough for us to be able to crystallize the complex for x-ray diffraction studies.

Diphtheria toxin is a complex proenzyme with the ability to introduce an intact fragment of itself through a cell membrane. 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 will learn how an aqueous protein can interact with and insert into membranes and how this interaction permits the transmembrane movement of the intact peptide. During this year, we grew crystals of the monomeric toxin from a solution containing polyethylene glycol, NaCl, and LiCl. The crystals are of the orthorhombic space group $P222_1$ and diffract to 2.5 A resolution. Using synchrotron radiation (Orsay, France), we collected 40% of a complete 2.5 A data set from native crystals. We are now in the process of completing that data set, then plan to collect data from good heavy-atom isomorphores derivatives of the crystal to obtain phase information. When the phases have been determined with sufficient accuracy, we plan to calculate a map of the native toxin and begin structural interpretations from that map.

Δ^5-3-ketosteroid isomerase catalyzes a step necessary for synthesis of steroid hormones. This enzyme binds tightly to certain steroids, which act as compe-

titive inhibitors of the enzyme, thus mimicking certain features of steroid receptor proteins. By determining the atomic structure of Δ^5-3-ketosteroid isomerase, we should discover important general features of protein-steroid interactions.

The crystal structure of this enzyme has been determined at 6 Å resolution. Difference-Fourier analysis of the structure of an enzyme-inhibitor complex at 6 Å resolution has also shown the location and some characteristics of the active center of the enzyme. Diffraction data were collected at 2.5 Å resolution with a synchrotron x-ray source (Orsay, France). The approximate phases were determined for the data with a mean figure of merit of 0.55. The electron density map of the enzyme calculated with these 3.0 Å structure factors has been used to initiate a cyclic process of phase improvement using the methods of density modification.

Calmodulin. This molecule has been studied primarily through x-ray and neutron small angle scattering, which allow us to determine molecular shapes and sizes in solution--their biologically functional state. During the past year, we placed significant emphasis on the development of new detectors for x-ray and neutron scattering. A small angle x-ray instrument utilizing a novel self-scanning photodiode array detector was constructed and is now in routine use. The electronics for time-resolved small angle x-ray scattering were tested out at the Stanford Synchrotron Radiation Laboratory; real time calculations were made for radius of gyration and forward scattering from 10 msec.

During this year, also, the time-of-flight neutron small angle diffractometer, which we developed, was used for a variety of biological studies and is now routinely available at IPNS-I (a national users' facility) as a research instrument. A new data acquisition system was installed to improve counting rates and to improve ease of operation for outside investigators.

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13. REGULATION OF CELL GROWTH

H. E. Kubitschek, Principal Investigator
W. W. Baldwin* and R. A. Ward†

This program has been concerned with the elucidation of cellular and molecular mechanisms common to both prokaryotes and eukaryotes in the regulation of cell growth. A primary objective is to test the hypothesis that cell mass increases in linear fashion during the cell cycle. This hypothesis is based upon earlier studies of growth of synchronous cultures and single cells and is supported by our earlier observations of constant rates of uptake of precursors into bacteria and in yeast.

Conventional methods of cell biology and biochemistry are used in studies of the growth and division cycle. Increases in cell mass or volume and division of cells are the fundamental features that define the cell growth cycle and establish cell ages at which other cell properties are measured. Synchronous cultures were produced by the Mitchison-Vincent method of separating cells by velocity sedimentation in density gradients, then selecting the smallest cells for synchronous culture. Increase in cell volume was determined with a precision Coulter counter-Davidson analyzer cell-sizing system of high resolution, and cell size and DNA content were determined by flow cytometry. Values of cell buoyant densities, required to calculate cell mass from volume, were obtained by equilibrium centrifugation in Percoll gradients.

Last year we reported in collaboration with Dr. M. R. Loken (University of Chicago), that cell buoyant densities were constant during the growth and division cycle in three different mouse cell lines, as well as in Escherichia coli. Furthermore, buoyant densities of E. coli were also found to be independent of cell growth rate, an unexpected finding because cell size and RNA content are known to increase markedly at rapid growth rates. These results provide evidence for the operation of a regulatory system that maintains cell buoyant density remarkably constant at all cell ages and growth rates.

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†Fall 1983 Student Research Participant, Austin College, Sherman, TX.

Recently, we examined further implications of these earlier results. We found, in agreement with other reports, that cell buoyant density does not remain constant during the cell cycle of the budding yeast Saccharomyces cerevisiae but varies cyclically. This result suggested that buoyant density constancy might be maintained only in cells that divide by equatorial fission. We obtained support for this hypothesis in experiments with the large fission yeast Schizosaccharomyces pombe; we found that buoyant densities in these cells remained constant during the cell cycle, as well as at different growth rates. Thus, our results show that buoyant densities are maintained constant during growth of three very different kinds of cells that divide by equatorial fission: bacteria, yeast, and mammalian cells.

Our discovery of the highly constant buoyant cell density in these prokaryotic and eukaryotic cells provides evidence for a new density regulatory system that operates in cells that divide by equatorial fission.

In addition, we have obtained evidence that cell buoyant densities of E. coli are under the control of the cell osmoregulatory system. We were able to increase cell buoyant densities by increasing the osmolarity of growth media, by addition of either sodium chloride or sucrose. Cell growth rate and its variability also were dependent upon osmolarity. Our results to date lead to the following general conclusions:

1. Buoyant density constancy in widely divergent types of cells (bacteria, yeast, mammalian) provides further evidence for similar mechanisms of control of growth in prokaryotes and eukaryotes and suggests that the regulatory system for buoyant density has been conserved during evolution.

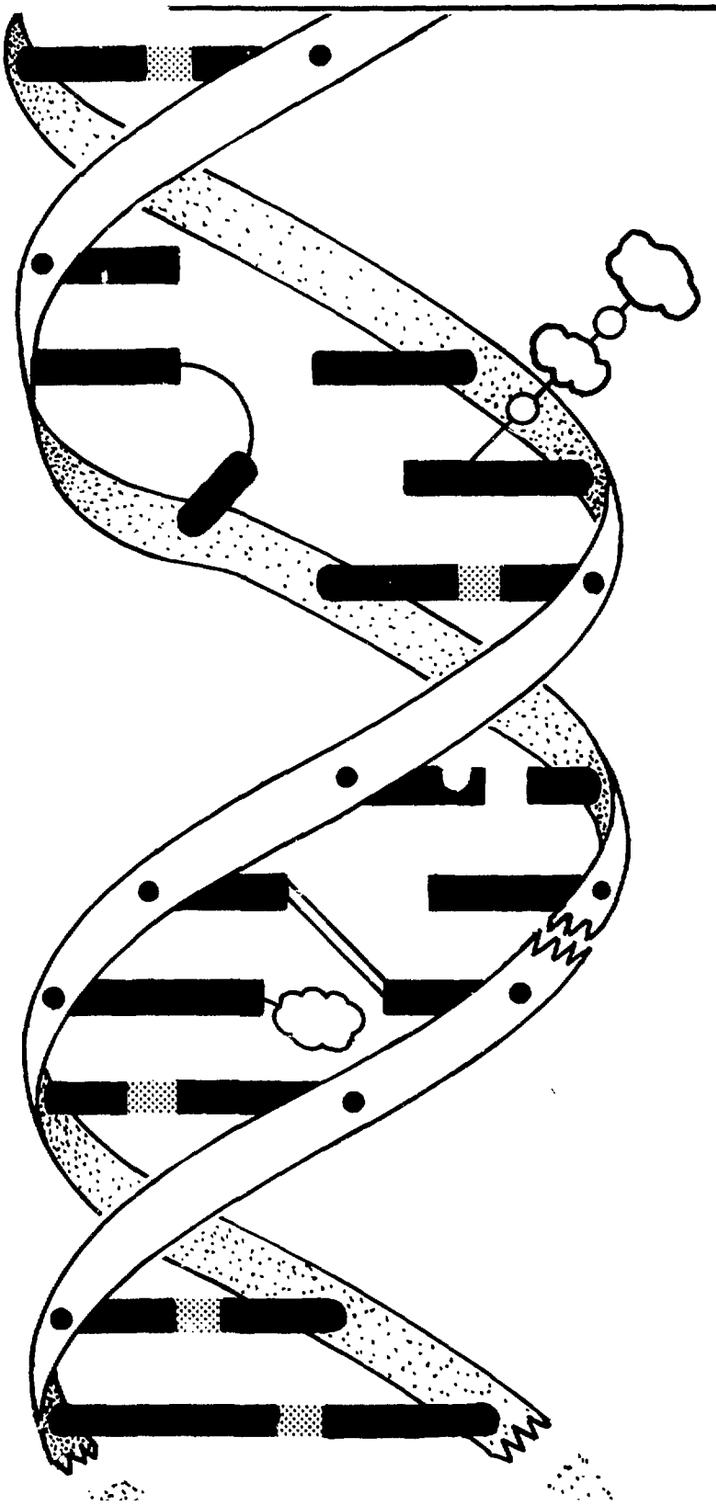
2. Since buoyant cell density is invariant during the cycle, density variation cannot provide signaling functions for growth and division, as has been proposed by others.

3. Cell mass is proportional to cell volume throughout the cell cycle in bacteria, yeast, and murine cultures. Because cell volume increases linearly during the cell cycle in E. coli, buoyant density constancy in these cells requires linear mass increase, i.e., linear growth.

4. Finally, we have identified the control system in E. coli as a part of the osmoregulatory system. It is likely that similar systems are involved in buoyant density control in other cells.

Our evidence for linear mass increase suggests that, despite the large numbers of genes involved in cell growth processes (such as the synthesis of amino acids, nucleic acids, membranes, and cell walls), the basic phenomena of cell growth are controlled primarily by only a few general processes during the growth cycle.

Toxicology



Dr. Charles F. Ehret is shown below with one of the animals he uses in the circadian regulation experiments described in Chapter 17.

14. CHEMICAL TOXICOLOGY

C. A. Reilly, Jr., Principal Investigator
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The Chemical Toxicology Group forms one part of a multidisciplinary research program that addresses the risks to human health from exposure to complex mixtures of chemicals (see Chapter 15 for a description of another component of the program). Investigations from this program and others have revealed that the toxicological effects of mixtures of chemicals are sometimes different from those predicted by adding the responses to the individual compounds making up the mixture. At present, information on interactions within complex mixtures that may be responsible for modifying toxicity is limited. Thus, the overall objectives of our research are to systematically evaluate the physical and chemical interactions among the components of simple mixtures of organic compounds and various class fractions of complex organic mixtures and to elucidate the unifying principles governing the ways in which the interactions modify toxicologic responses. Emphasis is placed on mixtures derived from energy production, but the principles are thought to apply to all toxicologic assessments. Because of the large spectrum of organic chemical classes found in materials produced during conversion of fossil fuels, results of experiments with these materials can provide models for health effects following exposure to chemicals from a broad range of sources, including toxic waste.

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The observations from health effects research on coal conversion by-products at this laboratory and from similar studies at other national laboratories provide a logical base for a study of the definitive principles of toxic chemical interactions. As a first step, we are conducting studies with two fundamentally different approaches. In one approach, the mutagenic potential of many types of mixtures is measured under a variety of conditions in vitro using bacterial (Ames Salmonella) and mammalian cell systems (Chinese hamster ovary and human P_3 teratocarcinoma). Such tests allow the study of interactions in reasonably well-defined systems with limited and identifiable variables. They also provide advantages of speed and economy. These results assist in the design of animal studies to further extend the observations. In the other approach, the initial experiments are conducted with whole animals to identify the effects in complete biological systems. We are studying dermal carcinogenesis in the SKH hairless mouse either following chronic exposure or by an initiation/promotion assay. These experiments are then followed by the in vitro studies designed to elucidate the basis for the altered responses.

Chemical characterization studies of a number of tar materials consistently showed in both bacterial and mammalian cell tests that fractionation by liquid-liquid pH partitioning into acidic, basic, and neutral fractions results in a highly mutagenic basic fraction (less than 4% of the total mass but 30-50% of the total mutagenicity), a mutagenic neutral fraction (60-70% of the mass and 30-50% of the total mutagenicity), and a nonmutagenic acidic fraction (25-30% of the mass). The sum of mutagenic activities of the fractions in this assay was roughly equivalent to that determined for the unfractionated material. In contrast, inhibition of carcinogenesis was observed in the unfractionated tar in a dermal carcinogenicity study in SKH mice. The relative carcinogenicity of the whole tar and its three fractions was determined following chronic application of the material to the dorsal skin three times per week for up to 26 weeks. The amount of each fraction used was equivalent to its fractional concentration in the whole tar. Mice treated with the neutral fraction developed tumors significantly sooner than those treated with either the acidic or basic fractions or the unfractionated tar. One hundred percent of the animals treated with the neutral fraction developed squamous cell carcinomas, with a median time to tumor appearance of 9 weeks. Similarly, 100% of the mice exposed to the unfractionated (crude) tar developed skin tumors but these tumors appeared later (median time to first tumor of 16 weeks), and only 46% of the tumors were malignant. The basic and acidic fractions were significantly less carcinogenic than either the crude tar or its neutral fraction. Animals treated with the basic fraction had a tumor incidence of 90% with a 43-week median time to tumor appearance (21% of the tumors were squamous cell

carcinomas). In the acidic subfraction treatment group, only 40% of the mice developed tumors (24% of the tumors were squamous cell carcinomas). A number of possible explanations exist for the greater carcinogenicity of the neutral fraction; thus, elucidation of the mechanism of inhibition in the unfractionated material is the main goal of current studies. Additional experiments are in progress that will determine the carcinogenicity of stoichiometrically recombined tar fractions (e.g. basic + neutral, acidic + neutral, etc.), thus providing insights into the mechanism of the inhibition.

Additional studies are also in progress to help determine the potential carcinogenicity of low concentrations of highly mutagenic aromatic amines. We observed in the mouse dermal carcinogenicity study that the basic fraction (in which the dominant mutagens are aromatic amines) was not significantly carcinogenic. This finding is not surprising as aromatic amines are primarily noted for systemic effects (i.e. induction of colon and urinary bladder tumors). The basic fraction is currently being evaluated for its potential as a colon or bladder carcinogen following dermal exposure in Syrian hamsters.

Results from the mammalian toxicology studies performed within this project, combined with those from the other studies in the multidisciplinary program will provide the supportive evidence for unifying principles of interactions of components within complex mixtures affecting the toxicity of the mixtures. Knowledge of these principles will help form the basis for quantitative predictions of the potential risks associated with exposures to defined complex organic chemical mixtures, irrespective of the source.

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15. CARCINOGEN IDENTIFICATION AND METABOLISM

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The studies reported here are one component of a multidisciplinary research program (see Chapter 14) designed to examine the health risks involved in exposure of humans to complex mixtures of chemicals resulting from energy conversion processes. The specific objectives of this component of the program are (1) to better define adverse health effects of environmental chemicals by studying toxicologic responses to mixtures of chemicals and (2) to further characterize the metabolic activation of aromatic amines, especially for compounds of toxicologic importance. For these studies, we are isolating and characterizing subclasses of chemical compounds from environmentally significant complex mixtures and developing analytical and biochemical approaches to study aromatic amine metabolism.

Chemical and Toxicologic Characterization of Coal Conversion Materials

Liquid partitioning procedures were used to systematically isolate classes of chemicals from by-products of coal-conversion processes. The toxicologic activities of the chemical class fractions were measured by assays for dermal and systemic carcinogenicity in mice and by assays of cytotoxicity and mutagenesis in bacterial (Salmonella typhimurium) and mammalian (hamster and human) cells. Chemical class fractions of special interest because of their source and genotoxicity were selected for further fractionation with liquid chromatographic procedures. These procedures included ion-exchange, reverse-phase, and normal - phase high - performance liquid chromatography (HPLC). We

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developed and validated the ion-exchange procedures and adopted other procedures to suit our needs. The liquid chromatographic fractions were assayed for bacterial mutagenesis, and the most active fractions were further resolved chemically.

The chemical composition of the mutagenic fractions and subfractions was determined primarily by gas chromatography (GC) and gas chromatography-mass spectrometery (GC-MS), with additional information provided by their liquid chromatographic characteristics and their UV-visible absorption spectra. To process the resulting data, we developed a computer-based system, which greatly facilitated qualitative GC analysis.

Our studies this year have shown that polycyclic aromatic hydrocarbons are among the principal mutagenic and carcinogenic components of by-products from coal-conversion processes. We also showed that although aromatic amines are minor constituents, they are responsible for a major portion of the mutagenicity of the mixtures as measured in bacteria. Of these, highly mutagenic 3-ring aromatic amines (aminoanthracenes and aminophenanthenes) and their alkyl homologs are major contributors to the mutagenic activity. Therefore, we are studying the metabolism and metabolic activation of representative 3-ring aromatic amines (as described in the following section).

Metabolism of 2-aminoanthracene (2AA)

As for most chemical carcinogens, the activity of aromatic amines in complex chemical mixtures depends on their metabolic conversion to reactive products that bind covalently to cellular constituents, including DNA. Evidence obtained by a variety of approaches indicates that aromatic amines are metabolically activated by N-oxidation. This evidence has been primarily obtained from comprehensive studies of aminonaphthalene, aminobiphenyl, and aminofluorene. However, the metabolism of aminophenanthrenes and aminoanthracenes has received little attention, despite their potent mutagenic and carcinogenic activities. Thus, we have begun to examine the metabolic activation of 2-aminoanthracene.

Rat liver microsomes were isolated from untreated animals and animals treated with either phenobarbital, 3-methylcholanthrene, or a mixture of polychlorinated biphenyls (PCBs). These agents increase the levels of various forms of membrane-bound enzymes (monooxygenases) that are responsible for the initial step in metabolic activation of chemical carcinogens. The conversion of 2AA to metabolites by the microsomes was detected and measured by HPLC under conditions devised to stabilize 2AA and its metabolites. The mutagenicity of HPLC fractions was measured in the mutagenesis assay with Salmonella typhimurium strain TA98 in the absence of exogenous enzymes for metabolic activation. Several 2AA

metabolites were detected and isolated, most of which are as yet unidentified. The major mutagenic metabolite was tentatively identified as 2-nitrosoanthracene, based on its chromatographic behavior, its reactions with glutathione, its mass spectrum, and an understanding of the fate of other aryl amines. This elusive metabolite has not been previously isolated or chemically synthesized.

When the concentration of 2AA in the enzymatic reaction mixture was 50-100 μM, the formation of 2-nitrosoanthracene was induced about 8-fold by pretreatment of the rats with either 3-methylcholanthrene or polychlorinated biphenyls. These agents apparently increased the levels of a monooxygenase active for N-oxidation of 2AA. When similar reaction conditions (50 μM 2AA) were used to compare the effectiveness of the various microsome preparations to convert 2AA to mutagens as detected in the Salmonella mutagenesis assay, the microsomes from rats treated with 3-methylcholanthrene and PCB were about 8-fold more active than microsomes from untreated and phenobarbital-treated rats. This finding was consistent with HPLC measurements also showing an 8- to 10-fold increase in the formation of the mutagenic metabolite 2-nitrosoanthracene. However, when the 2AA concentration in the mutagenesis assay was lower (< 2 μM), as is typical for mutagenesis screening, the activities of the microsomes from the treated and untreated rats for metabolic activation differed in the opposite sense. We are exploring the basis for this concentration-dependent difference in relative activities. Obviously, interpretation of results from comparative mutagenesis assays requires critical consideration of biochemical variables.

Comparative Mutagenicity and Carcinogenicity of Aryl Amines

Energy-related complex mixtures contain alkyl homologs of aromatic amines that are also likely to contribute to mutagenicity and carcinogenicity. These include alkyl homologs of 3-ring aromatic amines, e.g., aminoanthracenes. The mutagenic and carcinogenic activities of the 2-ring compounds 2-aminonaphthalene and 4-aminobiphenyl are enhanced by the presence of an ortho-methyl substituent, but the effect of methyl substitution of aminoanthracenes and aminophenanthrenes has not been described. We found that methyl substitution did not markedly enhance the mutagenicity of 2AA. These studies also showed that the mutagenic activities of aminoanthracenes and aminophenanthrenes had a greater than 100-fold range depending on the position of the amino group.

The mutagenic activity of 2AA is at least 20-fold greater than that for 1-aminoanthracene. We also compared the activities of 1- and 2-aminoanthracene in an hepatic initiation/promotion assay (see Chapter 1) in which newborn rats were treated with the test compounds to determine the initiating activity of the compounds, followed by promotion with dietary

phenobarbital at weaning. Foci of altered cells were detected histochemically by the presence of gamma-glutamyl transpeptidase. Neither of the compounds tested was significantly active. The lack of activity may be due to the low efficiency of the microsomes from the livers of neonatal rats for metabolic activation of 1- and 2-AA as determined in subsequent experiments.

In conclusion, our identification of aryl amines as major contributors to mutagenesis by coal conversion materials has led to studies of aromatic amine metabolism. The present studies focus on isomers and homologs of aminoanthracenes with emphasis on refinement of experimental approaches useful for study of aromatic amines in general. Our work on the characterization of the principal types of mutagens/carcinogens in complex coal conversion materials has led to work directed toward better understanding the toxicology of chemical mixtures in a more general sense, with the aims including (1) better interpretation of the results for bioassays of mixtures and (2) a better understanding of the principal types of chemical and biochemical interactions that affect the combined toxicologic effects of chemical mixtures. This effort involves isolation and characterization of well-defined mixtures for use in studying the toxicology of complex mixtures in animal and cellular systems.

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16. 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 in rats and (2) cadmium-induced alterations in kidney function and skeletal structure in mice. 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 for determining 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.

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Metablism and Toxicity of Compounds of Cadmium and Lead

Influence of exposure to lead on vitamin D metabolite concentrations during pregnancy and lactation. This study was conducted to determine whether exposure to lead during pregnancy and lactation in rats reduces concentrations of circulating $1,25(OH)_2$vitamin D. 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. Serum concentrations of $1,25(OH)_2$vitamin D and calcium are reduced in children with elevated levels of lead in blood [Rosen et al., N. Eng. J. Med. 302, 1128 (1980)].

Results of our study showed that lead exposure during pregnancy and lactation produced clear alterations in pathways for heme metabolism. Compared to the control dams receiving no lead, dams receiving diets containing 250 or 500 ppm lead exhibited threefold increases in free erythrocyte protoporphyrin concentrations and 11% decreases in hematocrits on day 14 of lactation. In the same animals, concentrations of vitamin D metabolites were not altered by lead exposure. Concentrations of $1,25(OH)_2$vitamin D in the plasma of dams on day 14 of lactation were 80-100 pg/mL in both lead-exposed and control groups. These concentrations in dams on day 14 of lactation were increased 5-fold over those in nonpregnant rats in which plasma $1,25(OH)_2$vitamin D concentrations were 17-20 pg/mL with or without dietary lead exposure. In rats, therefore, even when blood lead levels were high enough to cause changes in heme metabolism, lead did not block the rise in $1,25(OH)_2$vitamin D concentrations that accompanied the onset of lactation.

Two conclusions can be drawn from our study: (1) During lactation, the mother and her offspring develop toxic responses to lead at exposure levels that do not affect nonpregnant animals (see last year's annual report, ANL-83-40) and (2) the nonpregnant adult rat, the pregnant/lactating adult rat, and the neonatal rat pup are all less susceptible than the human child to the effects of lead on concentrations of $1,25(OH)_2$vitamin D.

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 is examining for the first time the role of cadmium exposure during multiple pregnancies on the generation of 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 household" diet (deficient in certain vitamins, minerals, and fat). One-half of the females were bred for six consecutive pregnancy/lactation cycles; the remaining females were nonpregnant controls. After six successive cycles, mice were oophorectomized to bring about hormonal changes characteristic of the postmenopausal period in humans.

Following are results of our study to date: (1) Female mice exposed to the deficient "Itai-Itai household" diet and 50 ppm cadmium showed decreases in reproductive parameters (fertility, litter size, weaning weight of pups) that did not occur in mice on a sufficient diet. (2) No decreases in reproductive parameters occurred in mice given 5 ppm cadmium, regardless of type of diet. (3) Multiparous mice exposed to 50 ppm cadmium showed decreases in total calcium content and calcium/dry weight ratios of femurs, changes possibly characteristic of Itai-Itai disease. The effects of oophorectomy of multiparous mice, to bring about postmenopausal changes, remain to be determined.

Gastrointestinal (GI) Absorption of Plutonium and Other Actinides

Our current aims are (1) to develop a method for determining the GI absorption of plutonium and uranium in the adult baboon that does not involve sacrificing the animal and (2) to use this method to determine the GI absorption of plutonium and uranium under specific conditions of feeding and fasting. The "nonsacrifice" method requires simultaneous administration of two isotopes of a given actinide, one orally and one intravenously [DeGrazia et al., J. Lab. Clin. Med. 66, 822 (1965)]. GI absorption values are calculated from separate analyses of blood, urine, and/or biopsies of bone or liver. In our first methods-validation study, we administered multiple isotopes of plutonium and uranium to one baboon according to the following protocol: ^{236}Pu and ^{233}U administered orally to the fasted baboon on day 0; ^{238}Pu and ^{236}U administered intravenously at the same time to the same baboon on day 0; sacrifice, day 32.

Results indicate that for uranium, the pathways for tissue deposition and urinary excretion for the orally administered ^{233}U were essentially identical to those for the intravenously administered ^{236}U. For plutonium, results differ in one important way: The percentage of the absorbed dose appearing in urine for the orally administered ^{236}Pu (80%) was considerably greater than that for the intravenously administered ^{238}Pu (11%). The pattern of deposition of plutonium among tissues of the body (liver vs. skeleton), and within the skeleton itself (femur vs. rib vs. skull), however, were the same for the orally and intravenously administered isotopes of plutonium. The possibility is being investigated that contamination of urine by oral ^{236}Pu in feces accounted for the apparently greater urinary excretion of ^{236}Pu than ^{238}Pu.

Using the dual isotope method to determine GI absorption values for our one baboon, we have found excellent agreement between calculated and analytical results. Uranium absorption was 1.4% of the oral dose, as determined at sacrifice by summation of that retained in the baboon (0.2%) and that excreted in urine (1.2%). The calculated value was 1.3% ± 0.3% (mean ± SD, $n = 7$) based on separate analyses of urine, liver, and bone samples. Plutonium absorption in the baboon was 0.020% of the oral plutonium, as determined by summation of that retained in the baboon (0.018%) and that excreted in day 1 urine (0.002%). The calculated value was 0.021% ± 0.004% (mean ± SD, $n = 6$) based on separate analyses of blood, urine, liver, and bone samples. Once validated, this nonsacrifice method of determining GI absorption by analysis of urine, blood, and/or biopsy samples after dual isotope administration will have clear advantages over the procedure involving analysis of all excreta, as well as the entire baboon at sacrifice.

Directions for the near future include (1) identification of an animal species whose pathways for vitamin D metabolism are sensitive to lead exposure to study the mechanism by which lead alters concentrations of $1,25(OH)_2$vitamin D, (2) histological and microradiographic examination of bone changes in oophorectomized, multiparous mice following exposure to cadmium, and (3) validation of the nonsacrifice method for determining GI absorption values of plutonium and uranium in additional baboons, with eventual application of this method to the neonatal nonhuman primate.

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D. P. Peterson, E. S. Moretti, and
M. I. Spaletto
Adsorption of Plutonium, Lead, and Cadmium to Mouth Surfaces during Oral Administration to Mice
Health Phys.

17. Neurobehavioral Chronobiology: Circadian Toxicology and Electric Field Effects

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The major objective for each of the two studies described here is to show that alterations of the characteristic waveforms of biological oscillations of circadian (about a day) and ultradian (less than a day) periodicities can be used diagnostically to judge the capacity of potentially toxic agents and conditions to cause neurological and behavioral disorders. Emphasis is on the actions of agents and conditions that have escaped traditional screening techniques but that nevertheless pose neurobehavioral health hazards.

For these studies, rats are singly housed in automated systems in which inputs (food, light, toxicants) are strictly controlled and from which physiological information is continuously monitored 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). Baseline "signatures" are obtained, then the experimental agent is applied. An altered signature (circadian dyschronism) in the presence of a toxic agent or an electric field indicates potential performance abnormalities. Such losses or derangements of the normal circadian rhythm waveform have been associated with a variety of neurologic and psychopathic disorders.

Circadian Dyschronogenic Action of Lithium: Is Lithium a Zeitgeber?

In previous studies, rats that had been chronically exposed to lithium at levels considerably below the LD_{50} showed circadian dyschronism within 5-10 days of

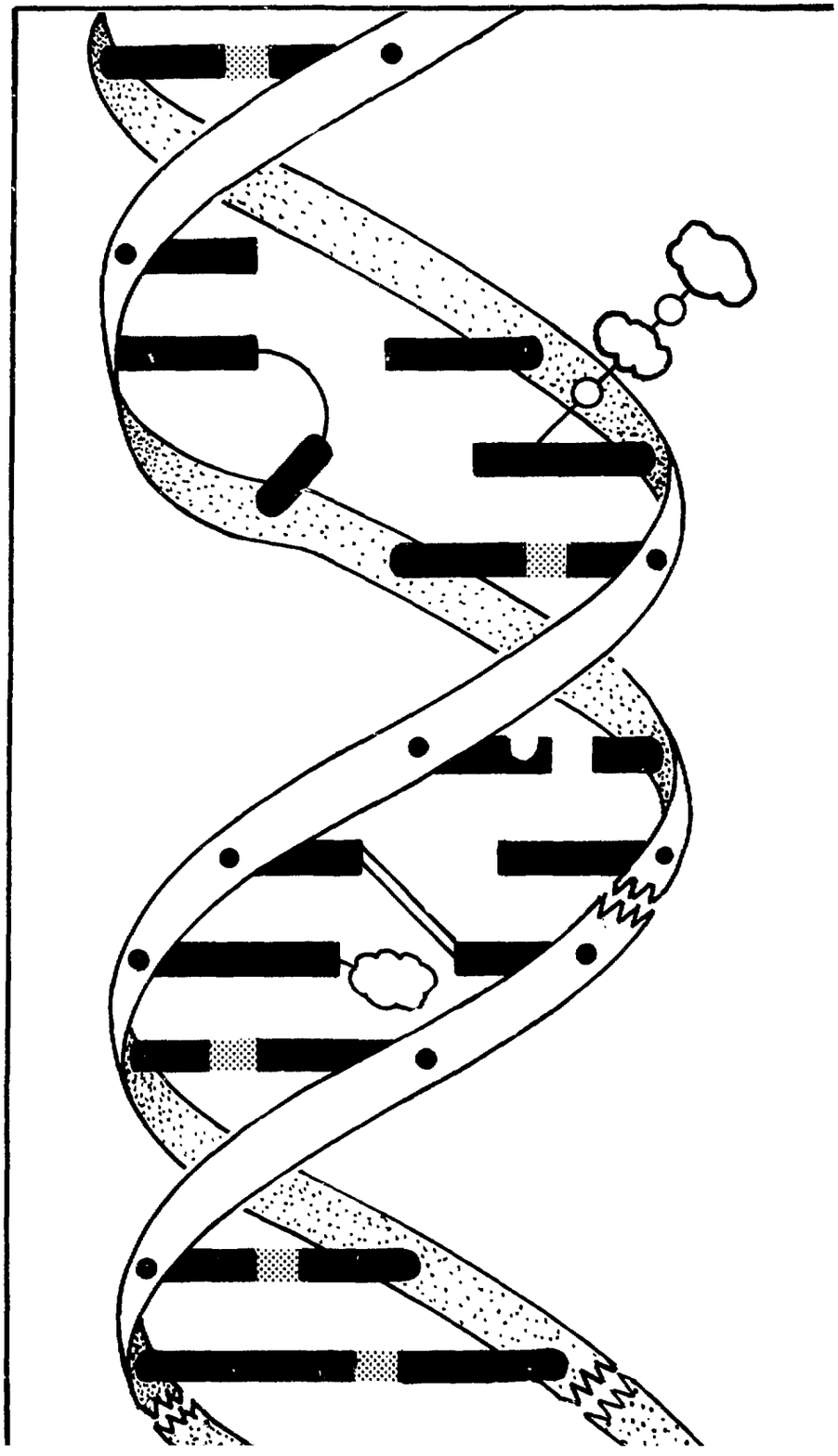
*Faculty Research Participant, Western Michigan University, Kalamazoo.
†Spring and Summer Student Research Participant, Purdue University, Ft. Wayne, IN.
§Spring and Summer Student Research Participant, University of South Dakota, Rapid City.
**Spring and Summer Student Research Participant, St. Xavier College, Chicago, IL.

the onset of exposure. In the study reported here, we examined whether a single injection of lithium could act as a zeitgeber (agents that phase-shift circadian clocks) depending on the phase in the circadian cycle at which it was administered. Accordingly, protocols appropriate to the determination of circadian phase response curves were used: circadian-phase-specific ("time-of-day" specific) injections were given during "free run" (when the animals are deprived of circadian entrainment signals) to one or another of eight groups of well-entrained animals at 3-hour intervals throughout the course of a single day, and the animals were maintained in free run for 6 or 7 days. Immediately following injection, no matter when in the cycle it was given, lithium was decisively dyschronogenic with significant reductions in circadian amplitude and apparent phase shifts of the core temperature rhythm over 2-3 days; however, after this period, in 58 out of 60 animals, normal circadian rhythms resumed; these rhythms were indistinguishable in phase from those for saline-injected controls. In conclusion, although a single injection of lithium administered at therapeutic levels appears to be significantly dyschronogenic, it exerts no long-lasting effect on the circadian phases of either temperature or activity rhythms and, therefore, cannot be regarded as a circadian zeitgeber or as one of the typical chronobiotically active drugs (such as theophylline, α-methyl-p-tyrosine, or levodopa).

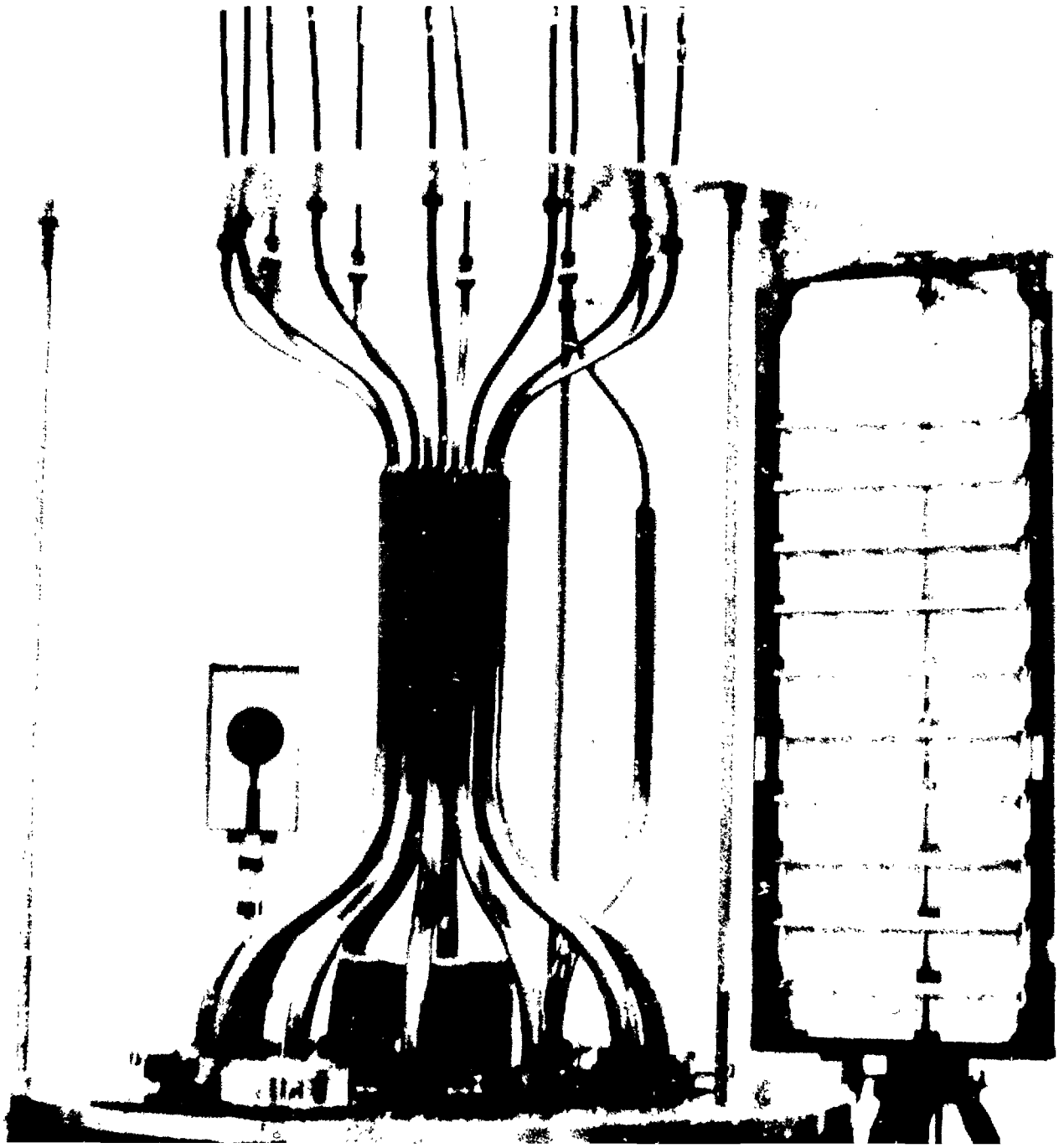
The Induction of Circadian Phase Shifts in Small Rodents by High-Strength 60-Hz Electric Fields

This study is designed to focus on a key finding of an earlier series of experiments, namely, that 60-Hz electric fields cause circadian phase delays in the white-footed mouse Peromyscus leucopus and thus are circadian zeitgebers. Our field-exposure and data acquisition facilities were modified and reconstructed to enhance the precision and sensitivity of activity and respiration measurements, and a series of experiments is under way designed to answer the following questions: In P. leucopus, do electric fields also cause phase advances? What is the full shape of the generalized circadian phase response curve? Can comparable phase shifts be induced in another rodent species, the laboratory white rat? Under what conditions can dyschronism be induced and/or prevented following arousal by 60-Hz electric fields in small rodents? These questions are all highly relevant to the evaluation of the health risk to humans of high-strength electric fields because 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.

Division Support Facilities, Services, and Activities



The photograph below was taken in the high-level *gamma room* (F-005) and shows mice, individually housed in 1-pint polyethylene containers, being irradiated with gamma rays from a ^{60}Co source.



18. SUPPORT FACILITIES

Animal Facilities

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

Our 40,000 ft^2 accredited animal facility, a major resource of the Division, consists of four major components: (1) dog kennels with a capacity for approximately 700 dogs; (2) two building wings, Wings E and Q, consisting of 47 animal rooms for housing small laboratory animal species; (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, a "hot room" for handling radioactive materials, and an injection room for treating animals with toxic chemicals and carcinogens.

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

During 1983, approximately half of the staff members of the Division were involved in various studies in which more than 18,000 rodents and other laboratory animal species were used.

The supporting facilities for the husbandry and clinical monitoring of the resident animals include the following (responsible personnel in parentheses):

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

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

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

Clinical pathology laboratory (D. V. Tolle)

Hematology laboratory (D. V. Tolle)

Necropsy laboratory (T. E. Fritz)

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

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

An important aspect of the success of any animal research program is the health of its animal population. To assure a supply of high-quality animals, the facility has concentrated on breeding its own healthy, specific-pathogen-free animals. Most of the rodents, particularly mice, and all dogs are bred in the facility. The rodent breeding is managed by J. M. Angerman. The beagle breeding is supervised by C. M. Poole and W. G. Keenan, who are also responsible for the clinical and surgical care of the dogs.

The beagle colony, used primarily for low-level radiation studies in dogs (Chapters 8 and 9) has been a closed colony since 1960. Extensive computerized records are maintained under the supervision of D. E. Doyle on all aspects of the colony, including reproduction, genetics, hematology, pathology, and disease incidence. Computerized record systems are also maintained for the research programs in which rodents are used.

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

The Animal Care Specialists during 1983 were the following:

Claude C. Colegrove, Group Leader
William G. McDade, Jr., Group Leader
Adrian J. Cordova
Mose Burrell
Charles J. Fowler
Rita Fuchs
MaDonna Gandalovics
Carrey R. Herringer
Robert A. Herringer
Betty L. McKay
Richard A. Santarelli
Leon L. Stewart
Diane M. Thomas
Jose A. Torres
Joseph N. Wilson

Radiation Facilities

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

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

In addition to the radiation sources maintained in the Division for the irradiation of biological materials, two other neutron sources in the Chicago area are available to the staff for cell biology studies comparing neutron beams with different characteristics: the linear accelerator at the Cancer Therapy Facility of the Fermi National Accelerator Laboratory (which produces neutrons with a mean energy of 25 MeV, $\underline{p}^+ \rightarrow Be$) and the Franklin McLean Research Institute, The University of Chicago (which produces neutrons with a mean energy of 8 MeV, $\underline{d}^+ \rightarrow Be$).

^{60}Co Gamma Radiation

F-005 Gamma Room Radiation Field Dosimetry

Cavity configuration. Exposure rates from 500 to 16,000 R/minute can be obtained with ± 2% variation within volumes as large as 25 cm^3.

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

F-101 Gamma Room Radiation Field Dosimetry

Panoramic configuration. Exposure rates from 1 mR/minute to 30 mR/minute can be achieved by varying distance and attenuation. Up to 500 small rodents may be irradiated simultaneously at four different exposure rates with a worst-case deviation of 7% from the mean. Animals are maintained in individual plastic cages, with food and water, on a frame with an N x 5 matrix. N varies from 4 to 8 depending on the frame distance from the source.

F-114 Gamma Room (intermediate) Radiation Field Dosimetry

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

X and Y Gamma Rooms Radiation Field Dosimetry

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

Fission-Spectrum Neutron Radiation

JANUS Reactor Radiation Field Dosimetry

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

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

X-Radiation

General Electric Maxitron 250-kVp x-ray machine, a general Divisional facility.

Westinghouse Flurodex "300" 130-kVp x-ray unit equipped with a Machlett, Dynamax-40, rotating anode, dual focal spot tube. This unit is used for clinical and diagnostic purposes for small animals and dogs.

Norelco Müller MG "300" 300 kVp x-ray machine used for radiobiological research and radiotherapy.

19. EDUCATIONAL ACTIVITIES

Postgraduate Training

During 1983, a total of 13 postdoctoral appointees and research associates contributed to the research programs of the Division. Seven of these were new appointees in 1983, while five finished their assignments during the year.

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

James M. Burcham	Iowa State University	C. Peraino
Chong-Hwan Chang	University of Pittsburgh	M. Schiffer
Michael L. Cunningham	University of Arizona	C. A. Reilly, Jr.
Kaoru Kiguchi	Jikei University School of Medicine, Tokyo, Japan	E. Huberman
Peter A. Lagocki	University of Chicago	Y. E. Rahman
Etienne A. Malvoisin	University of Louvain, Brussels, Belgium	E. Huberman
Ingrid Marshall	University of Karlsruhe, Karlsruhe, West Germany	D. Grahn
Shin-ichi Murao	Kobe University, Kobe, Japan	E. Huberman
Carol T. Oravec	Ohio State University	E. Huberman
Michael T. Short	University of Illinois at the Medical Center	M. Schiffer
Ido Simon	Weizmann Institute of Science, Rehovot, Israel, and the University of Iowa	E. Huberman
Mark S. Swanson	University of Nebraska at the Medical Center	D. A. Haugen
Pappannan Thiyagarajan	University of Illinois at the Medical Center	C. S. Borso
Robert L. Wells	Colorado State University	A. Han

Faculty Research Participations

Argonne and the Division have long involved faculty members from colleges and universities and staff members from industrial laboratories in Argonne activities. During 1983, three Visiting Scientists, nine 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. All of these appointments enable faculty members to participate in the research activities of the Laboratory to broaden their perspectives; they also allow university professors and Argonne scientists to develop rapport and explore mutual interests. In addition, the Division of Educational Programs in 1983 initiated a pilot program involving summer research participation by high school teachers; 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 during 1983, their schools, and their staff sponsors are as follows:

William W. Baldwin*	Indiana University School of Medicine, Northwest Center	H. E. Kubitschek
William E. Boernke†	Nebraska Wesleyan University	C. Peraino
Mukul C. Data§	Tuskegee Institute	T. M. Seed
Dona J. Fowler§	Western Michigan University	C. F. Ehret
Randolph Grayson§	California Polytechnic State University	E. Huberman
Carey W. Hanly†	University of Illinois	M. Schiffer
Richard M. Hyslop§	Illinois Benedictine College	C. S. Borso
Donald K. Jasper§	Illinois Institute of Technology	T. E. Fritz
Florence S. Lewis§	Cheyney State College	E. Huberman
Pamela M. Moehring§	Mount Mary College, Milwaukee	M. J. Peak
J. Emory Morris*	State University College of New York	C. Peraino
Frank H. Pascoe§	College of St. Francis	N. G. Anderson
Mark E. Pennington**	Waubonsie Valley High School	N. L. Anderson
Juarine Stewart§	Atlanta University	D. A. Haugen
Marvin Stodolsky†	Bosphorus University, Istanbul, Turkey	E. Huberman
Bartlett D. Whelton*	Eastern Washington University	M. H. Bhattacharyya

*Scientist in Residence (Faculty Research Leave at Argonne Appointee).
†Visiting Scientist.
§Faculty Research Participant.
**High School Faculty Research Participant.

Graduate Programs

Three graduate students were Laboratory Graduate Participants working in the Division 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 at Chicago Circle	M. H. Bhattacharyya
Frederick J. Temmel	University of Iowa	C. A. Reilly, Jr.
Mary A. Turner	University of Missouri	M. J. Peak

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 1983, one student held this appointment in the Division:

Greg S. Spicer	Texas Technical University	N. G. Anderson

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 1983, a total of 35 college undergraduates received training in the Division of Biological and Medical Research through the Spring, Summer, and Fall Undergraduate Research Participation Programs, sponsored by the Division of Educational Programs. The students, their schools, and their staff supervisors are listed below:

Spring Program

Ralph H. Clover	Purdue University	C. F. Ehret
David A. Eberhard	University of South Dakota	C. F. Ehret
Helen M. Kane	Immaculata College	T. M. Seed
Mary E. Minkwitz	College of St. Francis	M. J. Peak
Marian F. Papiernik	St. Xavier College	C. F. Ehret
David L. Redman	Ohio Wesleyan University	A. Han
Sally J. Schroeter	Nebraska Wesleyan University	H. E. Kubitschek
Charles M. Tomkins	Morris Brown College	F. R. Kirchner
Jane M. Toomey	Stonehill College	M. Bhattacharyya
Rachael L. Weiderhold	Olivet Nazarene College	F. R. Kirchner

Summer Program

Gwen Campbell	Salem College	T. M. Seed
Ralph H. Clover*	Purdue University	C. F. Ehret
David A. Eberhard*	University of South Dakota	C. F. Ehret
William Finley	Lehigh University	B. A. Carnes
Michael Goodrich	Calvin College	F. S. Williamson
Mary J. Hampton	Ft. Valley State College	D. V. Tolle
Thomas Hickok	Macalester College	F. R. Kirchner
Jennifer Hines	Oberlin College	H. E. Kubitschek
Diane Kremkow	Lewis University	F. R. Kirchner
Susan Loess	Bradley University	D. V. Tolle
Cheryl Ann Martin	College of the Holy Cross	Fritz/Lombard
Richard McMasters	Northwestern University	M. Schiffer
Mary E. Minkwitz	College of St. Francis	M. J. Peak
Marian F. Papiernik*	St. Xavier College	C. F. Ehret
William Reisdorf	University of Michigan	C. S. Borso
Kathy Rowlen	Grand Valley State College	F. R. Kirchner
Benedict Shen	Bowdoin College	M. Schiffer
Robert Sikorski	Pennsylvania State University	M. J. Peak
Lisa Williams	Bradley University	M. H. Bhattacharyya

Fall Program

Gregory W. Culen	St. Xavier College	F. R. Kirchner
Beth W. Dunlop	Alma College	F. R. Kirchner
Mark W. Elliott	Carroll College	T. E. Fritz
James P. Greer	Bellarmine College	F. R. Kirchner
Gregory A. Grose	Carroll College	C. F. Ehret
John M. Holland	Carroll College	C. K. Hill
Rebecca M. Jacobs	Oberlin College	M. J. Peak
Michael J. Morris	University of Alabama	C. F. Ehret
Robert A. Ward	Austin College	H. E. Kubitschek

*Also participant in the Spring Undergraduate Research Participation Program.

20. DIVISIONAL SEMINARS IN 1983

The Division of Biological and Medical Research Seminar Committee for 1983 consisted of M. J. Peak (chairman), M. H. Bhattacharyya, C. A. Jones, C. A. Reilly and T. M. Seed. T. M. Seed served as host for the in-house speakers.

The General Seminar Program for 1983 consisted of a combination of in-house and vistiting speakers, selected on the basis of recommendations from BIM staff members. In addition, a number of informal seminars in specialized subjects were held during the year and during the summer period when the general seminars were not scheduled; some of these informal talks are indicated in the following listing.

Visiting Speakers

Dr. John T. Leith, Division of Biology and Medicine, Brown University, Providence, RI

Radiological Aspects of Tumor Heterogeneity

January 13, 1983

Dr. Alfred Nisonoff, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA

Regulation of the Immune Response Mediated Through Idiotypic Determinants

January 27, 1983

Dr. Bernard S. Strauss, Department of Microbiology, Cummings Life Sciences Center, the University of Chicago, Chicago, IL

In Vitro Models of Base Substitution Mutagenesis: A Role for the Polymerase

February 10, 1983

Dr. Toshio Kuroki, Department of Pathobiochemical Cell Research, The Institute of Medical Science, the University of Tokyo, Tokyo, Japan

Can Two-Stage Carcinogenesis in Mouse Skin Be Extrapolated to Human Skin?

February 21, 1983

*Dr. **Leonard H. Augenlicht**, Sloan-Kettering Institutes for Cancer Research, Rye, NY
Gene Expression in Cancer
March 3, 1983

Dr. Moshe Shifrine, Laboratory for Energy-Related Health Research, University of California, Davis
Seasonal Variation in Immunity of Dogs and Man
March 10, 1983

†**Dr. David J. Grdina**, Department of Experimental Radiotherapy, M. D. Anderson Hospital, Houston, TX
Radiobiological and Cellular Heterogeneity of Tumor Cell Subpopulations
March 15, 1983

Dr. Joseph Aloni, Department of Genetics, The Weizmann Institute of Science, Rehovot, Israel
Regulatory Elements in the Control of Eucaryotic Gene Expression
April 4, 1983

Dr. Kate E. Carr, Department of Anatomy, University of Glasgow, Glasgow, Scotland, UK
The Effects of Radiation Quality, Hyperthermia, and Other Modulating Factors on the Morphological Responses of Postirradiated Gastrointestinal Tissues
April 28, 1983

Dr. Kevin McEntee, Department of Biological Chemistry, University of California Medical Center, Los Angeles
Gene Regulation and DNA Repair in Prokaryotes and Eukaryotes
June 2, 1983

Dr. R. J. Michael Fry, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN
Ultraviolet Radiation Carcinogenesis
June 9, 1983

Dr. Ann R. Kennedy, Harvard School of Public Health, Boston, MA
Factors Influencing X-ray Induced Neoplastic Transformation <u>In Vitro</u>
June 16, 1983

*Informal seminar.
†Dr. Grdina joined the Division in September 1983.

Dr. R. M. Tyrrell, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland
Lethal and Mutagenic Action of Solar Radiation on Cultured Human Cells
June 23, 1983

Dr. Sushilkumar G. Devare, Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD
Simian Sarcoma Virus: Genome Organization and Transforming Gene Product
June 24, 1983

*Dr. Anne Rybicki, Department of Biochemistry, The University of Chicago, Chicago, IL
Membrane Cytoskeleton Interaction in the Red Blood Cells
August 25, 1983

Dr. Linda L. Randall, Biochemistry Department, Washington State University, Pullman
Export of Protein in Escherichia coli
September 22, 1983

Dr. Thomas G. Ebrey, Department of Physiology and Biophysics, University of Illinois, Urbana
Proton Pumping in the Purple Membrane of Halobacterium halobium
October 6, 1983

Dr. Steven Kennel, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN
Monoclonal Antibodies and Some of Their Applications
October 13, 1983

Dr. Robert J. Cousins, Department of Food Sciences and Human Nutrition, University of Florida, Gainesville
Molecular Aspects of Copper and Zinc Metabolism
November 10, 1983

Dr. Morris Pollard, Director, Lobund Laboratory, University of Notre Dame, Notre Dame, IN
Life in a Sterile Environment
December 1, 1983

*Informal seminar.

Dr. Reggie H. Stevens, Radiation Research Laboratory, Department of Radiology, University of Iowa, Iowa City
Radiation Equivalency: A Quantitative Measure for Carcinogenicity
December 8, 1983

In-House Speakers

Dr. Marianne Schiffer, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Three-Dimensional Structure of Immunoglobulin Light Chains
January 20, 1983

Dr. Peter A. Lagocki, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Effects of Charge and Size on the Uptake of Unilamellar Liposomes by Macrophages
February 17, 1983

Dr. Colin K. Hill, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Oncogenic Transformation of Mammalian Cells: Role of Radiation Quality
March 24, 1983

Dr. Charles S. Borso, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Small Angle X-Ray and Neutron Scattering: Instrumentation and Information Content
April 21, 1983

Dr. Herbert E. Kubitschek, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Control of Cell Growth and Density in Prokaryotes and Eukaryotes
May 19, 1983

Dr. Douglas Grahn, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Somatic and Genetic Effects of Low Dose Exposure to Fission Neutrons and Cobalt-60 Gamma Irradiations
May 26, 1983

Dr. Mary Ann Turner, Division of Biological Sciences, University of Missouri-Columbia, and Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Biological Effects of Near Ultraviolet Radiation
September 8, 1983

Dr. Edwin M. Westbrook, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Structural Studies of Natural Antimicrobial Proteins Isolated from Blood Leukocytes
October 20, 1983

Drs. Meyrick J. Peak and Bruce A. Carnes, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
The Ames Salmonella Mutagenicity Assay
November 17, 1983

Dr. N. Leigh Anderson, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
Detection of Mutations on 2-D Gels
December 15, 1983

21. OUTSIDE TALKS BY DIVISIONAL STAFF DURING 1983*

Dr. Norman G. Anderson

High-Resolution Two-Dimensional Electrophoresis Image Analysis and Data Base Management for the Human Protein Index
Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, PA
March 9, 1983

An ISO-DALT Electrophoresis System with Reduced Buffer and Current Requirements
Electrophoresis '83, Tokyo, Japan
March 10, 1983

The Interdependence of Technological Innovation and Knowledge Acquisition in the Biological Sciences
Department of Clinical Biochemistry, University of Toronto, Toronto, Canada
April 11, 1983

The Human Protein Index: Towards the Complete Analysis of Human Cells
Department of Clinical Biochemistry, University of Toronto, Toronto, Canada
April 12, 1983

High Resolution Two-Dimensional Electrophoresis
International Conference on New Developments in Clinical Laboratory Instrumentation, Robert S. First, Inc., Dusseldorf, Germany
November 28, 1983

Diagnosis and Treatment in the Future
Monterey Med-Tech Financial Forum, Monterey, CA
December 5, 1983

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

Image Analysis and Interactive Database Manipulation Applied to Two-Dimensional Maps of Human Proteins
National Computer Graphics Association Meeting, Chicago, IL
June 30, 1983

Gene Regulation Systems of Human Cells: Enumeration by Protein Indexing
Conference on Technology Impact: Potential Directions for Laboratory Medicine, New York, NY
September 21, 1983

Dr. N. Leigh Anderson

Data Reduction for the Human Protein Index
7th Annual Symposium on Computer Applications in Medical Care, Baltimore, MD
October 26, 1983

Dr. William W. Baldwin

Cell Density of Escherichia coli B/r during Exponential Growth
83rd Annual Meeting of the American Society for Microbiology, New Orleans, LA
March 9, 1983

Changes in Density and Mean Cell Volume during Growth in Escherichia coli B/r
83rd Annual Meeting of the American Society for Microbiology, New Orleans, LA
March 10, 1983

Dr. Maryka H. Bhattacharyya

Determination of a Gastrointestinal Absorption Factor for Plutonium in Man: An Evaluation of Existing Data
31st Annual Meeting of the Radiation Research Society, San Antonio, TX
March 2, 1983

Dr. Charles S. Borso

Determination of Biological Structures with Resonant Neutron Small Angle Scattering Utilizing a Pulsed Neutron Source
Winter Meeting of the American Crystallographic Association, Columbia, MO
March 15, 1983

Dr. Bruce A. Carnes

Estimation of the Ridge Constant: An Approach Based on the Condition Index
1983 Annual Meeting of the American Statistical Association, Toronto, Canada
August 15, 1983

Mr. Don E. Doyle

A Direct Data Entry System for Differential Leukocyte Counts
Southern Wisconsin-Chicago American Association for Laboratory Animal Science Meeting, Milwaukee, WI
November 16, 1983

Dr. Charles F. Ehret

Stay the Course While Traveling
The Staywell Executive Program, Control Data Corporation, Minneapolis, MN
January 11, 1983

Neurobehavioral Chronobiology and Circadian Regulation
Winter Meeting of the Academy of Integrated Medical Studies and the Gesell Institute for Human Development, Lenox, MA
February 5 and 6, 1983

New Approaches to the Problems of Jet Lag and Shift Work for Air Crews and Flight Attendants
Executive Committee Conference, International Flight Attendants Association, Chicago, IL
March 16, 1983

Future Perspectives for the Application of Chronobiological Knowledge in Occupational Work Scheduling
Invited Witness, Congressional Hearings on Biological Rhythms and Shift Work Scheduling, Investigations and Oversight Subcommittee of the Committee on Science and Technology, U. S. House of Representatives, Washington, DC
March 24, 1983

Circadian Connections to Coherent Processes in the Functioning of the Brain
Third Annual Neuroscience Conference, Maharishi International University, Fairfield, IA
May 10, 1983

The Importance of Meal Timing as a Circadian Zeitgeber in Laboratory Studies of Germ-Free Animals
University of Notre Dame, Notre Dame, IN
May 27, 1983

Introduction to the Role of Circadian Regulation in Mental Illness and in Sleep Disorders
Gordon Research Conference on Chronobiology, New London, NH
June 13, 1983.

High-Strength 60-Hz Electric Fields are Circadian Zeitgebers in Small Rodents
16th International Conference of the International Society for Chronobiology, Dublin, Ireland
July 27, 1983

Coherence and Correspondence in the Classical Composition of the Temporal and Structural Levels of Organization of Living Systems
Biennial Meeting of the Institute for Ultimate Reality and Meeting, University of Toronto, Toronto, Canada
August 17, 1983

Chronobiological Engineering: Circadian Connections to Recent Advances in Biotechnology
Department of Bioengineering, University of Illinois, Chicago, IL
October 25, 1983

Dr. Thomas E. Fritz

Radiation Factors Affecting Development of Myelogenous Leukemia in the Beagle
11th International Symposium of the International Association for Comparative Research on Leukemia and Related Diseases, Cambridge, England
July 6, 1983

Late Effects of Continuous Irradiation of Beagles: Hemopathology, Carcinogenesis, and Life Shortening
7th International Congress of Radiation Research, Amsterdam, The Netherlands
July 7, 1983

Hematopoietic Responses in Beagles Given Protracted Whole Body Gamma Irradiation: Leukemogenic Effectiveness of Varying Modalities of Exposure

12th Annual Meeting of the International Society for Experimental Hematology, London, England

July 13, 1983

The Preleukemic Syndrome in Radiation-Induced Canine Myelogenous Leukemia and Related Myeloproliferative Diseases

12th Annual Meeting of the International Society for Experimental Hematology; London, England

July 13, 1983

Late Effects of Protracted Whole Body Irradiation of Beagles by ^{60}Co Gamma Rays

22nd Annual Hanford Life Sciences Symposium, Richland, WA

September 28, 1983

Dr. Douglas Grahn

Recent Studies on the Effectiveness of High LET Radiations for the Induction of Genetic and Somatic Injury

Radiation Research Society Meeting, Symposium on Basic Issues in Radiation Protection, San Antonio, TX

February 27, 1983

Incidence of Abnormal Sperm as a Function of Low Doses of Gamma Rays or Fission Neutrons, Age, and Season

Environmental Mutagen Society Meeting, San Antonio, TX

March 3, 1983

Somatic and Genetic Effects of Low Doses of Fission Neutrons and ^{60}Co Gamma Rays

7th International Congress of Radiation Research, Amsterdam, The Netherlands

July 7, 1983

Biological Effects of Low-Doses of Fission Neutrons and ^{60}Co Gamma Rays

American Statistical Association Conference on Radiation and Health, Berkeley Springs, WV

July 11, 1983

Carcinogenic Effects of Fission Neutrons and ^{60}Co γ-rays

Workshop on Neutron Radiation Carcinogenesis, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN

September 19, 1983

Studies on Chronic Radiation Injury with Mice and Dogs Exposed to External Whole-Body Irradiation at the Argonne National Laboratory
22nd Annual Hanford Life Sciences Symposium, Richland, WA
September 27, 1983

Dr. David J. Grdina

Cell Separation and Radiation Biology
Alumni Symposium on Basic and Applied Radiobiology, University of Kansas, Lawrence
September 30, 1983

Tumor Heterogeneity and Radiation Biology: A Search for Prognostic Indicators of Tumor Response
Johns Hopkins University, Baltimore, MD
October 25, 1983

Application of Cell Separation Techniques to Experimental Chemotherapy and Radiotherapy
Johns Hopkins University, Baltimore, MD
October 25, 1983

Development of a Prognostic Indicator for Tumor Response
Oak Ridge National Laboratory, Oak Ridge, TN
November 9, 1983.

Dr. Antun Han

Role of Repair Processes and Radiation Quality in Neoplastic Transformation of Mammalian Cells
31st Annual Meeting of the Radiation Research Society, San Antonio, TX
March 1, 1983

Killing and Mutation of Mammalian Cells by Monochromatic Near-UV Light
11th Annual Meeting of the American Society for Photobiology, Madison, WI
June 27, 1983

Neoplastic Transformation Decreases after Irradiation with Multiple Fractions of ^{60}Co γ-Rays
7th International Congress of Radiation Research, Amsterdam, The Netherlands
July 7, 1983

Error-free Repair of Neoplastic Transformation Damage following Protracted Exposures to ^{60}Co γ-Rays
11th L. H. Gray Conference, Cellular Repair of Radiation Damage, Glasgow, Scotland
July 21, 1983

Dr. David A Haugen

Isolation and Identification of Mutagenic Components of Synfuel Materials
DOE Briefing on Coal Conversion, Germantown, MD
January 7, 1983

Dr. Colin K. Hill

Protr ed Exposures to Fission-Spectrum Neutrons Increase Neoplastic Trans. on
7th International Congress of Radiation Research, Amsterdam, The Netherlands
July 7, 1983

Error-prone Repair of Fission-Spectrum Neutron Induced Neoplastic Transformation
11th L. H. Gray Conference, Cellular Repair of Radiation Damage, Glasgow, Scotland
July 21, 1983

Radiation and Cell Transformation in vitro
Institute of Physics, University of Milan, Milan, Italy
October 12, 1983

Problems Associated with Transformation Studies
Institute of Physics, University of Milan, Milan, Italy
October 13, 1983

Dr. Eliezer Huberman

Control of Cell Differentiation in Cultured Human Cells by Tumor Promoting Agents
University of Maryland, School of Medicine, Maryland Cancer Program, Neoplastic Diseases Seminar Series, Baltimore, MD
January 6, 1983

The Control of Mutagenesis and Cell Differentiation in Cultured Mammalian Cells by Chemicals which Initiate or Promote Tumor Formation
Department of Genetics, Weizmann Institute for Science, Rehovot, Israel
April 19, 1983

The Control of Cell Differentiation in Cultured Human Cells by Tumor Promoting Agents
First International Conference on Carcinogenesis, American Cancer Society, Buffalo, NY
April 25, 1983

The Control of Cell Transformation, Mutagenesis, and Differentiation by Chemicals which Initiate or Promote Tumor Formation
International Workshop on the Principles of Environmental Mutagenesis, Carcinogenesis, and Teratogenesis, Shanghai, China
May 27, 1983

The Control of Cell Differentiation in Human Myeloid and T-Cell Lymphoid Leukemia Cells by Chemicals
Roswell Park Memorial Institute, Buffalo, NY
September 14, 1983

Dr. Carol A. Jones

Induction of Mutations in Human Cells by Solar Ultraviolet Radiation
Environmental Mutagen Society Meeting, San Antonio, TX
March 4, 1983

Activation of Chemical Carcinogens in an Intestinal-Cell-Mediated Mutagenesis Assay
American Association of Cancer Research, San Diego, CA
May 26, 1983

Dr. Herbert E. Kubitschek

Cell Growth and Density
DePaul University, Department of Biological Sciences, Chicago, IL
May 6, 1983

Constancy of Buoyant Density of E. coli and Cultured Murine Cells
Gordon Research Conference, Wolfeboro, NH
July 6, 1983

Dr. Shin-I. Murao

Control of Macrophage Cell Differentiation in Human Promyelocytic (HL-60) Leukemia Cells by 1,25-Dihydroxyvitamin D_3 and Phorbol-12-myristate-13-acetate (PMA)
42nd Annual Meeting of the Japanese Cancer Association, Nagoya, Japan
October 14, 1983

Dr. Meyrick J. Peak

Biological and Molecular Effects of Solar Ultraviolet Radiation
Dana Farber Cancer Institute, Boston, MA
May 7, 1983

Killing and Mutation by Solar UV in Bacteria
11th Annual Meeting of the American Society of Photobiology, Madison, WI
June 27, 1983

Dr. Carl Peraino

Relationship of Putative Preneoplastic Hepatocyte Foci to Hepatic Tumorigenesis
Symposium on the Role of Cocarcinogens and Promoters in Human and Experimental Carcinogenesis, Budapest, Hungary
May 16, 1983

Dr. Christopher A. Reilly, Jr.

Toxicological Evaluations of Complex Mixtures
U. S. Environmental Protection Agency, Chemical Research and Evaluation Branch, Washington, DC
February 24, 1983

Mr. John J. Russell

Plutonium Induced Depletion of Developing Ovarian Follicles in the Mouse
Annual Health Physics Meeting, Baltimore, MD
June 22, 1983

Dr. Marianne Schiffer

The Structure of λ_I-Bence-Jones Protein LOC at 5 Å
67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, IL
April 12, 1983

Dr. Thomas M. Seed

Hemopoietic Responses under Continuous Low Dose γ-Irradiation
Department of Radiation Therapy, Harvard University, Boston, MA
January 20, 1983

The Ultrastructure of Radiation-Induced Injury in Cells and Tissues: An Overview
Scanning Electron Microscopy/1983, Dearborn, MI
April 22, 1983

Radiation-Induced Aplastic Anemia
Scanning Electron Microscopy/1983, Dearborn, MI
April 22, 1983

Survival Patterns and Hemopathological Responses of Dogs under Continuous Gamma Irradiation
Radiobiological Institute, Rijswijk, The Netherlands
July 1, 1983

Acquired Radioresistance of Hematopoietic Progenitors under Continuous Low Daily Dose Gamma Irradiation
12th Annual Meeting of the International Society for Experimental Hematology; London, England
July 13, 1983

Hematopoiesis on Cellulose Ester Membranes (CEM). VII. Ultrastructure of Stroma of Marrow-Enriched Membranes with Trilineal Hematopoiesis
12th Annual Meeting of the International Society for Experimental Hematology; London, England
July 13, 1983

Dr. Michael T. Short

High Pressure Gel Permeation Chromatography Studies of Self-Associating Immunoglobulin κ_I Light Chains and Idiotype Anti-Idiotype Complexes
67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, IL
April 12, 1983

Dr. John Taylor

Argonne's Contribution to the USE Library for AP120B's
Array Meeting, Monterey, CA
April 27, 1983

Dr. Frederick J. Tremmel

Metabolic Activation of 2-Aminoanthracene in Neonatal Rats
67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, IL
April 12, 1983

PUBLICATIONS APPEARING DURING THE NOTED PERIODS

1982 (received in 1983)

Nyberg, D.
Sex, Recombination, and Reproductive Fitness: An Experimental Study Using Paramecium
Am. Nat. 120, 198-217 (1982)

1983

Cahill, A. L., D. Nyberg, and C. F. Ehret
Tissue Distribution of Cadmium and Metallothionein as a Function of Time of Day and Dosage
Environ. Res. 31, 54-65 (1983)

Ehret, C. F.
Biological Clocks and Shift Work Scheduling, Hearings before the Subcommittee on Investigations and Oversight, Committee on Science and Technology, House of Representatives, 98th Congress, First Session, March 23, 24, 1983 [No. 7], pp. 321-355

Ehret, C. F., and L. W. Scanlon
Overcoming Jet Lag
Berkley Publishing Corporation, NY, 1983

Nyberg, D., and P. Bishop
High Levels of Phenotypic Variability of Metal and Temperature Tolerance in Paramecium
Evolution 37, 341-357 (1983)

Rosenberg, R. S., P. H. Duffy, G. A. Sacher, and C. F. Ehret
Relationship Between Field Strength and Arousal Response in Mice Exposed to 60-Hz Electric Fields
Bioelectromagnetics 4, 181-191 (1983)

Accepted as of June 30, 1984

Cahill, A. L., and C. F. Ehret
Chronobiological Consequences of Various Shiftwork Schedules
Proceedings of the 15th International Conference of the International Society of Chronobiology, Minneapolis, MN, 9/13-18/81

Ehret, C. F., C. Peraino, J. C. Meinert, and K. R. Groh
Circadian Manifestations of Barbituate Habituation, Addiction, and Withdrawal in the Rat
Intl. J. Chronobiol.

Dr. Mary A. Turner

Evidence that Near-Ultraviolet Radiation (NUV 313 nm) Does Not Induce the recA Gene

11th Annual Meeting of the American Society for Photobiology, Madison, WI

June 28, 1983

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22. PROFESSIONAL AFFILIATIONS

Joint and Adjunct College or University Appointments

During 1983, 16 staff members held a total of 18 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, 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, Northern Illinois University, DeKalb, 1979-present

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

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

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

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

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

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

- **Eliezer Huberman**, Professor of Cell Biology and Molecular Genetics, 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, Northern Illinois University, DeKalb, 1971-present

- **Meyrick J. Peak**, Adjunct Professor, Northern Illinois University, DeKalb, 1982-present

- **Carl Peraino**, Adjunct Professor, Northern Illinois University, DeKalb, 1971-present

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

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

- **Marianne Schiffer**, Lecturer, 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 during 1983 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

- **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 Committee 57, Task Group on Leukemia Risk
- **Thomas E. Fritz**, Member, Board of Directors, Illinois Cancer Council
- **Douglas Grahn**, Ordinary Member, International Council of Scientific Unions, Committee on Space Research, Sub-Commission F-2 on Radiation Biology of Interdisciplinary Scientific Commission F on Life Sciences as Related to Space
- **Douglas Grahn**, Council Member, National Council on Radiation Protection and Measurement
- **Douglas Grahn**, Member, National Council on Radiation Protection and Measurement

  Committee 40, Biological Aspects of Radiation Protection Criteria

  Committee 57, Internal Emitter Standards, Task Group 11 on Genetic Risk

  Committee 75, Guidance on Radiation Received in Space Activities

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

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

- **Carl Peraino**, Member, Research Committee, Illinois Division of the American Cancer Society

- **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

23. PUBLICATIONS FOR CALENDAR YEAR 1983

Journal Articles

Anderson, N. L., J. C. Wiltsie, C. Y. Li, K. E. Willard-Gallo, R. P. Tracy, D. S. Young, M. T. Powers, and N. G. Anderson
Analysis of Human Leukemic Cells by Use of High-Resolution Two-Dimensional Electrophoresis. I: Results of a Pilot Study
Clin. Chem. 29, 762-767 (1983)

Au, W. W., M. F. Callaham, M. L. Workman, and E. Huberman
Double Minute Chromatin Bodies and Other Chromosome Alterations in Human Myeloid HL-60 Leukemia Cells Susceptible or Resistant to Induction of Differentiation by Phorbol-12-myristate-13-acetate
Cancer Res. 43, 5873-5878 (1983)

Bhattacharyya, M. H.
Bioavailability of Orally Administered Cadmium and Lead to the Mother, Fetus, and Neonate during Pregnancy and Lactation: An Overview
Sci. Total Environ. 28, 327-342 (1983)

Bhattacharyya, M. H., B. D. Whelton, and D. P. Peterson
Gastrointestinal Absorption of Cadmium in Mice during Gestation and Lactation: II. Continuous Exposure Studies
Toxicol. Appl. Pharmacol. 66, 368-375 (1982)

Borso, C. S.
Implementation of Self-Scanning Photodiode Arrays for Scattering Applications Using Conventional and Intense X-Ray Sources
Trans. Am. Crystallogr. Assoc. 18, 141-148 (1982)

Cahill, A. L., D. Nyberg, and C. F. Ehret
Tissue Distribution of Cadmium and Metallothionein as a Function of Time of Day and Dosage
Environ. Res. 31, 54-65 (1983)

Collins, J. J., R. T. Lundy, and D. Grahn
A Demographic Model for Performing Site-Specific Health Risk Projections
Health Phys. 45, 9-20 (1983)

Cunningham, M. L., and B. R. Lokesh
Superoxide Anion Generated by Potassium Superoxide is Cytotoxic and Mutagenic to Chinese Hamster Ovary Cells
Mutat. Res. 121, 299-304 (1983)

Danon, M. J., C. S. Giometti, J. R. Manaligod, O. H. Perurena, and J. L. Skosey
Adult-Onset Nemaline Rods in a Patient Treated for Suspected Dermatomyositis
Arch. Neurol. 38, 761-766 (1981)

Deeg, H. J., R. Prentice, T. E. Fritz, G. E. Sale, L. S. Lombard, E. D. Thomas, and R. Storb
Increased Incidence of Malignant Tumors in Dogs after Total Body Irradiation and Marrow Transplantation
Int. J. Radiat. Oncol. Biol. 9, 1505-1511 (1983)

Dupere, S. L. F., S. Holland, S. Gawne, K. E. Cancelliere, B. A. Sedita, P. J. Dale, E. D. Jarrell, and T. E. O'Connor
Effects of Divalent Metal Cations on Composition and Neoplasia-specific Antigenicity of Chromatins
Cancer Res. 43, 4913-4919 (1983)

Gast, P., M. R. Wasielewski, M. Schiffer, and J. R. Norris
Orientation of the Primary Donor in Single Crystals of Rhodopseudomonas viridis Reaction Centres
Nature 305, 451-452 (1983)

Giometti, C. S., and M. J. Danon
Letter to the Editor of Neurology Regarding Fast to Slow Change of Myosin in Nemaline Rod Myopathy
Neurology 33, 1248-1249 (1983)

Giometti, C. S., M. J. Danon, and N. G. Anderson
Human Muscle Proteins: Analysis by Two-Dimensional Electrophoresis
Neurology 33, 1152-1156 (1983)

Grahn, D.
Genetic Risks Associated with Radiation Exposures during Space Flight
Adv. Space Res. 3, 161-170 (1983)

Grahn, D., C. H. Lee, and B. F. Farrington
Interpretation of Cytogenetic Damage Induced in the Germ Line of Male Mice Exposed for over 1 Year to ^{239}Pu Alpha Particles, Fission Neutrons, or ^{60}Co Gamma Rays
Radiat. Res. 95, 566-583 (1983)

Gray, S. H., C. F. Ainsworth, C. L. Bell, S. S. Danyluk, and M. MacCoss
Synthesis of Deoxyribonucleotidyl(3'-5')arabinonucleosides
Nucleosides and Nucleotides 2, 435-452 (1983)

Hagan, M. P., T. Matsushita, T. Bonura, and A. Shotola
Alkali-Labile Lesion and Uracil-DNA-Glycosylase-Sensitive Site Removal after BrdUrd and UVB Treatment of Chinese Hamster Cells
Photochem. Photobiol. 35, 371-377 (1982)

Harrison, H. H.
Improved Photography of Silver-Stained Two-Dimensional Electrophoresis Gels
Clin. Chem. 29, 1566-1567 (1983)

Haugen, D. A., and M. J. Peak
Mixtures of Polycyclic Aromatic Compounds Inhibit Mutagenesis in the Salmonella/Microsome Assay by Inhibition of Metabolic Activation
Mutat. Res. 116, 257-269 (1983)

Irving, C. S., C. L. Cooney, L. T. Brown, D. Gold, J. Gordon, and P. D. Klein
Microbial Fermentative Preparation of L-[$^{15}N_2$]Lysine and Its Tracer: Application to Serum Amino Acid Kinetic Studies
Anal. Biochem. 131, 93-98 (1983)

Jones, C. A., R. M. Santella, E. Huberman, J. K. Selkirk, and D. Grunberger
Cell Specific Activation of Benzo[a]pyrene by Fibroblasts and Hepatocytes
Carcinogenesis 4, 1351-1357 (1983)

Kirchner, F. R., P. F. Dunn, and C. B. Reed
Toxicologic and Physicochemical Characterization of High-Temperature Combustion Emissions
Aerosol Sci. Technol. 2, 389-400 (1983)

Kirchner, F. R., C. A. Reilly, Jr., D. M. Buchholz, and V. A. Pahnke, Jr.
Toxicological Effects on Mice following Inhalation Exposures to Fluidized-Bed Coal Combustor Fly Ash
Environ. Res. 32, 314-328 (1983)

Kubitschek, H. E., W. W. Baldwin, and R. Graetzer
Buoyant Density Constancy during the Cell Cycle of Escherichia coli
J. Bacteriol. 155, 1027-1032 (1983)

Kubitschek, H. E., and C. L. Woldringh
Cell Elongation and Division Probability during the Escherichia coli Growth Cycle
J. Bacteriol. 153, 1379-1387 (1983)

Larsen, R. P., D. M. Nelson, M. H. Bhattacharyya, and R. D. Oldham
Plutonium - Its Behavior in Natural Water Systems and Assimilation by Man
Health Phys. 44, 485-492 (1983)

Lau, E. H., E. A. Cerny, B. J. Wright, and Y. E. Rahman
Improvement of Iron Removal from the Reticuloendothelial System by Liposome Encapsulation of N,N'-bis[2-hydroxybenzyl]-ethylenediamine-N,N'-diacetic Acid (HBED): Comparison with Desferrioxamine
J. Lab. Clin. Med. 101, 806-816 (1983)

Ley, R. D., M. J. Peak, and L. L. Lyon
Induction of Pyrimidine Dimers in Epidermal DNA of Hairless Mice by UVB: An Action Spectrum
J. Invest. Dermatol. 80, 188-191 (1983)

MacCoss, M., J. J. Edwards, P. Lagocki, and Y.-E. Rahman
Phospholipid-Nucleoside Conjugates. 5. The Interaction of Selected 1-β-D-arabinofuranosylcytosine-5'-diphosphate-L-1,2-diacylglycerols with Serum Lipoproteins
Biochem. Biophys. Res. Commun. 116, 368-374 (1983)

MacCoss, M., J. J. Edwards, T. M. Seed, and S. P. Spragg
Phospholipid-Nucleoside Conjugates: The Aggregational Characteristics and Morphological Aspects of Selected 1-β-D-Arabinofuranosylcytosine 5'-Diphosphate-L-1,2-Diacylglycerols
Biochim. Biophys. Acta 719, 544-555 (1982)

Murao, S.-I., M. A. Gemmell, M. F. Callaham, N. L. Anderson, and E. Huberman
Control of Macrophage Cell Differentiation in Human Promyelocytic HL-60 Leukemia Cells by 1,25-Dihydroxyvitamin D_3 and Phorbol-12-myristate-13-acetate
Cancer Res. 43, 4989-4996 (1983)

Nyberg, D.
Sex, Recombination, and Reproductive Fitness: An Experimental Study Using Paramecium
Am. Nat. 120, 198-217 (1982)

Nyberg, D., and P. Bishop
High Levels of Phenotypic Variability of Metal and Temperature Tolerance in Paramecium
Evolution 37, 341-357 (1983)

Peak, J. G., M. J. Peak, and R. W. Tuveson
Ultraviolet Action Spectra for Aerobic and Anaerobic Inactivation of Escherichia coli Strains Specifically Sensitive and Resistant to Near Ultraviolet Radiations
Photochem. Photobiol. 38, 541-543 (1983)

Peak, M. J., and J. G. Peak
Use of Action Spectra for Identifying Molecular Targets and Mechanisms of Action of Solar Ultraviolet Light
Physiol. Plant. 58, 367-372 (1983)

Peak, M. J., J. G. Peak, and L. Nerad
The Role of 4-Thiouridine in Lethal Effects and in DNA Backbone Breakage Caused by 334 nm Ultraviolet Light in Escherichia coli
Photochem. Photobiol. 37, 169-172 (1983)

Rahman, Y. E., E. A. Cerny, E. H. Lau, and B. A. Carnes
Enhanced Iron Removal from Liver Parenchymal Cells in Experimental Iron Overload: Liposome Encapsulation of HBED and Phenobarbital Administration
Blood 62, 209-213 (1983)

Ranga, V., D. Grahn, and T. M. Journey
Morphologic and Phenotypic Analysis of an Outcross Line of Blotchy Mouse
Exp. Lung Res. 4, 269-279 (1983)

Raveh, D., and E. Huberman
A Microtiter Plate Assay for the Selection of 6-Thioguanine-Resistant Mutants in Chinese Hamster V79 Cells in the Presence of Phorbol-12-Myristate-13-Acetate
Mutat. Res. 113, 499-506 (1983)

Rosenberg, R. S., P. H. Duffy, G. A. Sacher, and C. F. Ehret
Relationship between Field Strength and Arousal Response in Mice Exposed to 60-Hz Electric Fields
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Schwartz, H. M., M. MacCoss, and S. S. Danyluk
Oxygen-17 NMR or Nucleosides. 2. Hydration and Self-Association of Uridine Derivatives
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Hemopathologic Predisposition and Survival Time under Continuous Gamma Irradiation: Responses Mediated by Altered Radiosensitivity of Hemopoietic Progenitors
Exp. Hematol. 10, Suppl. No. 12, 232-248 (1982)

Short, M. T., W. J. Eisler, Jr., and F. J. Stevens
Automated Fail-Safe Control System for Independent Operation of Two Radiation Shutters on a Rotating-Anode X-Ray Source
J. Appl. Cryst. 16, 359-360 (1983)

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Suppressive Action of Near-Ultraviolet Light on Ouabain Resistance Induced by Far-Ultraviolet Light in Chinese Hamster Cells
Mutat. Res. 119, 399-406 (1983)

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Numerical Measures of Two-Dimensional Gel Resolution and Positional Reproducibility
Electrophoresis 4, 338-346 (1983)

Thomson, J. F., F. S. Williamson, and D. Grahn
Life Shortening in Mice Exposed to Fission Neutrons and γ Rays: III. Neutron Exposures of 5 and 10 Rad
Radiat. Res. 93, 205-209 (1983)

Tolle, D. V., S. M. Cullen, T. M. Seed, and T. E. Fritz
Circulating Micromegakaryocytes Preceding Leukemia in Three Dogs Exposed to 2.5 R/Day Gamma Radiation
Vet. Pathol. 20, 111-114 (1983)

Tom, B. H., C. M. Macek, L. Raphael, J. Sengupta, E. A. Cerny, M. M. Jonah, and Y. E. Rahman
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Proc. Soc. Exp. Biol. Med. 172, 16-28 (1983)

Tuveson, R. W., J. G. Peak, and M. J. Peak
Single-Strand DNA Breaks Induced by 365 nm Radiation in *Escherichia coli* Strains Differing in Sensitivity to Near and Far UV
Photochem. Photobiol. 37, 109-112 (1983)

Utsumi, H., and M. M. Elkind
Caffeine-Enhanced Survival of Radiation-Sensitive, Repair-Deficient Chinese Hamster Cells
Radiat. Res. 96, 348-358 (1983)

Williams-Hill, D. M., and N. Grecz
Role of AP Endonuclease in DNA Breakage and Cell Inactivation of Escherichia coli Subjected to Mild Heat (52°C)
Mutat. Res. 107, 13-21 (1983)

Yotis, W. W., M. Zeb, P. C. Brennan, F. R. Kirchner, L. E. Glendenin, and C. D. Wu-Yuan
The Action of Selected Agents on the Accumulation of ^{18}F by Streptococcus mutans
Microbios 36, 21-32 (1983)

Books, Reports, and Proceedings

Ambrus, J. L., C. M. Ambrus, H. Gastpar, E. Huberman, R. Montagna, W. Biddle, S. Leong, and J. Horoszewicz
Antimetastatic and Antitumor Effect of Platelet Aggregation Inhibitors (ORNL)
Interaction of Platelets and Tumor Cells, Alan R. Liss, Inc., New York, NY, 1982, pp. 97-111

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Use of High-Resolution Two-Dimensional Electrophoresis in Human Health Effects Studies
Proceedings of the Oak Ridge Symposium on Health Risk Analysis, Gatlinburg, TN, October 27-30, 1980, pp. 357-365

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Electrophoresis '81, Allen and Arnaud, eds., Walter de Gruyter & Co., Berlin and New York, 1981, pp. 309-316

Anderson, N. G.
High-Resolution Protein Separation and Identification Methods Applicable to Virology
Current Topics in Microbiology and Immunology, Vol. 104, M. Cooper, P. H. Hofschneider, H. Koprowski, F. Melchers, R. Rott, H. G. Schweiger, P. K. Vogt, and R. Zinkernagel, eds., Springer-Verlag, Berlin, 1983, pp. 197-217

Anderson, N. L., J. Taylor, and N. G. Anderson
Image Analysis and Interactive Database Manipulation Applied to Two-Dimensional Maps of Human Proteins
Proceedings of the Fourth Annual Conference and Exposition of the National Computer Graphics Association, Chicago, IL, June 26-30, 1983, pp. 69-76

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24. STAFF AND FUNDING AGENCIES*

Carcinogenesis Section

Hepatocarcinogenesis

Regular Staff

C. Peraino (Senior Biochemist)
Principal Investigator

B. A. Carnes (Asst. Biostatistician)
J. A. Blomquist (Programmer)
V. A. Ludeman (Scientific Asst.)
A. M. Prapuolenis (Scientific Asst.)
J. J. Russell (Scientific Assoc.)
E. F. Staffeldt (Scientific Assoc.)

Temporary Staff

W. E. Boernke (Visiting Scientist)
J. M. Burchan (Postdoc. Appointee)
J. E. Morris (Scientist in Residence)

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, 1983, through June 1984. 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. vii).

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

Metal Carcinogenesis

Regular Staff

Y. E. Rahman (Senior Biologist)
Principal Investigator

E. A. Cerny (Scientific Asst.)

Temporary Staff

P. A. Lagocki (Postdoc. Appointee)

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"

Gene Expression and Carcinogenesis

Regular Staff

E. Huberman (Division Director)
Principal Investigator

C. A. Jones (Asst. Biochemist)
M. F. Callaham (Scientific Assoc.)
C. B. Henning (Scientific Assoc.)
B. A. Sedita (Scientific Asst.)

Temporary Staff

J. P. Hardwick (Postdoc. Appointee)
K. Kiguchi (Postdoc. Appointee)
E. A. Malvoisin (Postdoc. Appointee)
S.-I. Murao (Postdoc. Appointee)
C. T. Oravec (Postdoc. Appointee)
I. Simon (Postdoc. Appointee)
M. Stodolsky (Visiting Scientist)

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

C. K. Mill (Assistant Biologist)
Principal Investigator

M. M. Elkind (Sr. Biophysicist)
A. Han (Biophysicist)

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

NCI: 5 RO1 CA26984 "UV-X-Ray Interaction: Mutation and Transformation"

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

NCI: 5 R01 CA29940
"Damage-Repair Studies Related to Mammography"

Temporary Staff

R. L. Wells (Postdoc. Appointee)

NCI: 1 R01 CA33974
"Mutation-Transformation: Neutron Damage and Repair"

Low Level Radiation Section

Radiation Biology and Genetics

Regular Staff

D. Grahn (Senior Biologist)
Principal Investigator

D. J. Grdina (Radiation Biologist)
Principal Investigator

B. A. Carnes (Asst. Statistician)
K. Duggal (Pathologist)
W. E. Kisieleski (Chemist)
L. S. Lombard (Vet. Pathologist)
F. S. Williamson (Physicist)
B. F. Farrington (Scientific Asst.)
G. L. Holmblad (Scientific Assoc.)
J. L. Hulesch (Scientific Asst.)
V. A. Ludeman (Scientific Asst.)
A. R. Sallese (Scientific Asst.)
E. F. Staffeldt (Scientific Assoc.)
J. E. Trier (Engineering Asst.)
B. J. Wright (Scientific Assoc.)

Temporary Staff

I. R. Marshall (Postdoc. Appointee)
J. F. Thomson (Special Term Employee)

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"

NRC: Div. of Health, Siting, and Waste Management
ANL-8M456 "Relative Biological Effectiveness of Fission Neutrons and Gamma Rays at Occupational Exposure Levels"

Radiation Carcinogenesis	DOE:	Carcinogenesis (HA-02-02-01) ANL-60300 "Life Shortening and Tumor Induction by Low Dose Neutron and Gamma Irradiation
Regular Staff		
D. J. Grdina (Radiation Biologist) Principal Investigator	NCI:	7R01 CA37435 ANL-85622 "Radiation Response of Cells Separated from Tumors"
P. J. Dale (Scientific Asst.)		
Temporary Staff		
B. Nagy (Visiting Scientist)		
Radiation Toxicology	DOE:	Carcinogenesis (HA-02-02-01) ANL-63100 "Radiation Toxicity Studies in Dogs for Interspecies Comparisons"
Regular Staff		
T. E. Fritz (Vet. Pathologist) Principal Investigator	NCI:	Interagency Agreement (Y01-CO-0320) ANL-8D407 "Late Effects of Protracted Irradiation in Dogs"
K. Duggal (Pathologist)		
L. S. Lombard (Vet. Pathologist)		
C. M. Poole (Veterinarian)		
T. M. Seed (Biologist)		
D. V. Tolle (Asst. Biologist)		
D. E. Doyle (Scientific Asst.)		
G. L. Holmblad (Scientific Assoc.)		
L. V. Kaspar (Scientific Assoc.)		
W. G. Keenan (Scientific Assoc.)		
A. R. Sallese (Scientific Asst.)		
Radiation Hematology	DOE:	Carcinogenesis (HA-02-02-01) ANL-63105 "Hematopoiesis in Chronic Toxicity Studies"
Regular Staff		
T. M. Seed (Biologist) Principal Investigator		
L. V. Kaspar (Scientific Assoc.)		

Molecular Biology Section

Photobiology

Regular Staff

M. J. Peak (Microbiologist)
Principal Investigator

DOE: General Life Sciences (HB-01)
ANL-62201 "Cellular and Molecular Mechanisms of Radiation and Chemical Mutagenesis"

Molecular Anatomy

Regular Staff

N. G. Anderson (Sr. Physiologist)
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N. L. Anderson (Biophysicist)
C. S. Giometti (Asst. Biologist)
J. Taylor (Computer Scientist)
M. A. Gemmell (Scientific Asst.)
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

M. Schiffer (Biophysicist)
Principal Investigator

C. S. Borso (Asst. Biophysicist)
F. J. Stevens (Asst. Biophysicist)
E. M. Westbrook (Asst. Biophysicist)
C. F. Ainsworth (Scientific Asst.)
F. A. Westholm (Scientific Asst.)

Temporary Staff

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M. T. Short (Postdoc. Appointee)
P. Thiyagarajan (Postdoc. Appointee)
T.-S. Young (Postdoc. Appointee)

DOE: General Life Sciences (HB-01)
ANL-61200 "Protein Structure and Conformation"

NIAID: 1R01 AI19590
ANL-85565 "Study of Anti-Idiotypic Antibodies"

Mutagenesis

DOE: General Life Sciences (HB-01)
ANL-62201 "Cellular and Molecular Mechanisms of Radiation and Chemical Mutagenesis"

Regular Staff

H. E. Kubitschek (Sr. Biophysicist)
Principal Investigator

J. G. Peak (Asst. Biologist)
D. Williams-Hill (Scientific Asst.)

Temporary Staff

W. W. Baldwin (Scientist in Residence)
P. R. Reynolds (Postdoc. Appointee)

Toxicology Section

Chemical Toxicology

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

Regular Staff

C. A. Reilly, Jr. (Microbiologist)
Principal Investigator

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

D. A. Haugen (Biochemist)
F. R. Kirchner (Asst. Biologist)
M. J. Peak (Microbiologist)
S. S. Dornfeld (Scientific Asst.)
V. A. Pahnke (Scientific Asst.)
K. M. Suhrbier (Scientific Asst.)
D. Williams-Hill (Scientific Asst.)

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

Temporary Staff

M. L. Cunningham (Postdoc. Appointee)

Chemical and Biological Characterization

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

Regular Staff

D. A. Haugen (Biochemist)
Principal Investigator

K. M. Suhrbier (Scientific Asst.)

Temporary Staff

M. S. Swanson (Postdoc. Appointee)

Metal Toxicology	DOE:	Systems Damage (HA-02-02-03) ANL-61209 "Biological Effects of Metals"
Regular Staff		
M. H. Bhattacharyya (Biochemist) Principal Investigator	DOE:	Systems Damage (HA-02-02-03) ANL-61213 "Gastrointestinal Absorption of Actinides"
E. S. Moretti (Scientific Asst.)		
D. P. Peterson (Scientific Asst.)	NRC:	Div. of Health, Siting, and Waste Management ANL-8M447 "Reanalysis of Gastrointestinal Absorption Factors for Plutonium and Other Actinide Elements"
Temporary Staff		
B. D. Whelton (Scientist in Residence)		
Neurobehavioral Chronobiology	DOE:	Systems Damage (HA-02-02-03) ANL-61208 "Neurobehavioral Chronobiology and Toxicology"
Regular Staff		
C. F. Ehret (Sr. Biologist) Principal Investigator	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"
K. R. Groh (Scientific Assoc.)		

APPENDIX

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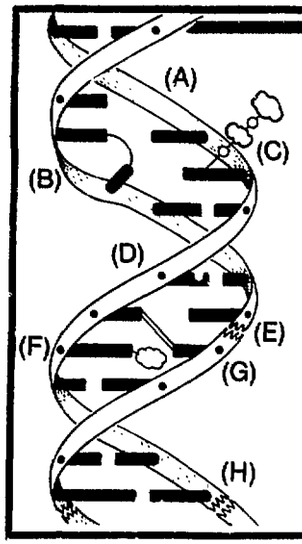

The cover design is a schematic representation of a short segment of a DNA molecule showing a number of the possible harmful lesions caused by environmental chemicals or radiations. The ultimate focus of many of the programs in the Division of Biological and Medical Research is the elucidation of the mechanisms by which these lesions are formed and the identification of various methods of protection and repair. These processes are investigated through a variety of techniques -such as two-dimensional electrophoresis, chromatography, sedimentation analysis, alkaline elution, cytofluorography, DNA sequencing, and the use of DNA probes and monoclonal antibodies. The lesions represented in the figure include (A) an apurinic site, (B) a pyrimidine adduct with a backbone distortion, (C) a peptide adduct, (D) a base change, (E) a single-strand break, (F) an adduct of a chemical carcinogen, (G) an interstrand crosslink, and (H) a double-strand break.

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