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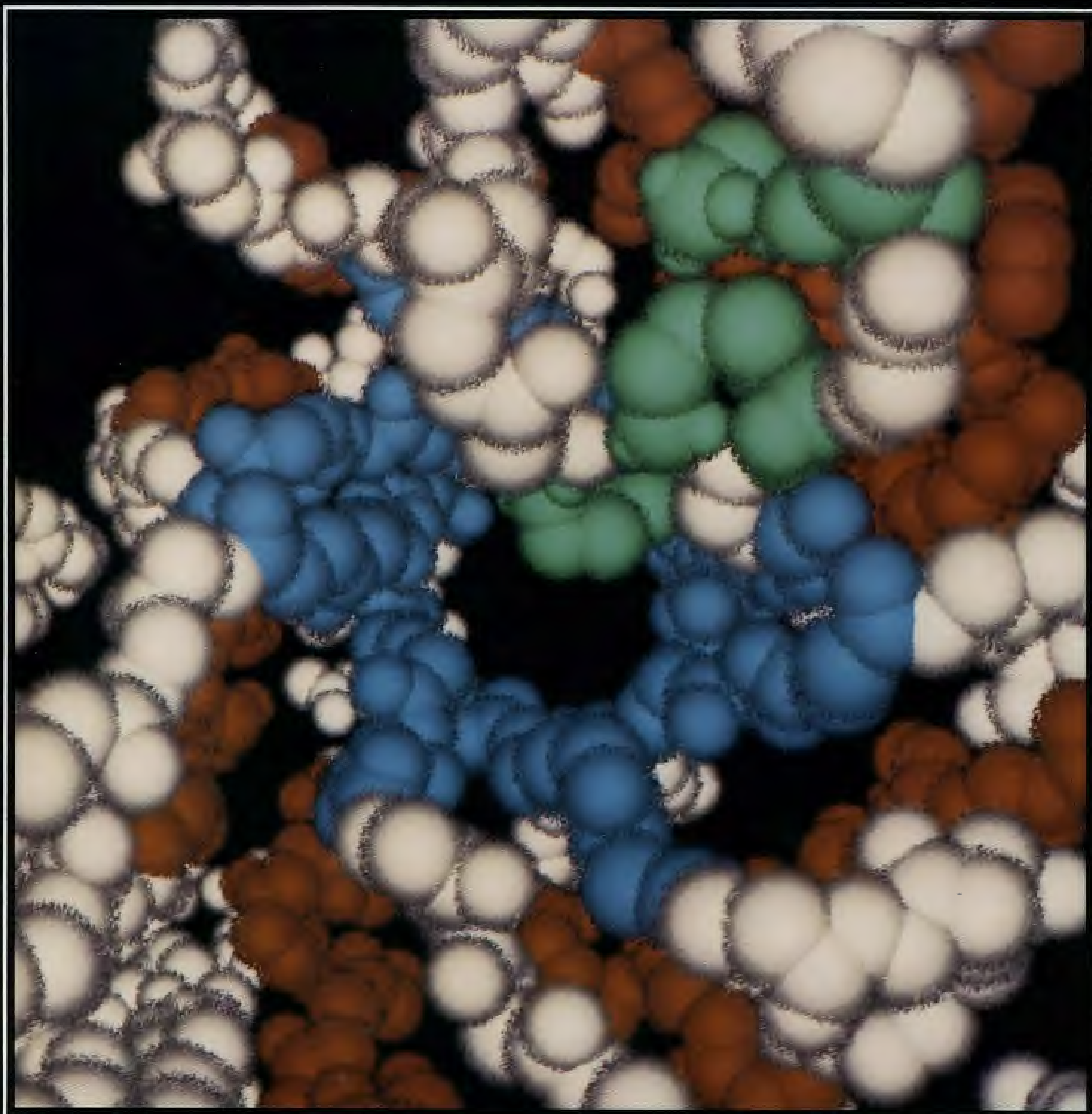

Life Sciences at ORNL

REVIEW

Volume 22 *Numbers Two and Three, 1989*

Oak Ridge National Laboratory is a multiprogram, multipurpose laboratory that conducts research in the physical, chemical, and life sciences; in fusion, fission, and fossil energy; and in energy conservation and other energy-related technologies.

ON THE COVER

This computer graphic, generated from crystallographic data provided by David Eisenberg of the University of California at Los Angeles, shows a portion of the active site of the carbon dioxide fixation enzyme, a cornerstone of all life. In this view, the barrel-shaped portion of the molecule has a blue inner surface and a red outer surface. Three amino acids, identified as crucial to catalysis by investigators in ORNL's Biology Division, are shown in green. Understanding this enzyme and improving its efficiency are goals of the Division's Protein Engineering Group. See the article on p. 60 in this special issue featuring the life sciences at ORNL.

ACKNOWLEDGMENTS

The *Review* staff gratefully acknowledges Bill Norris, for many of the photographs dealing with biology and environmental research; Lydia Corrill and Mike Aaron, for their many hours of invaluable editorial assistance; Vickie Conner, for designing the cover and departments; and Gloria Llanos and John Hart, of Hart Graphics in Knoxville, for design layout and production of the camera-ready pages. Special thanks are due Chester R. Richmond, ORNL's Associate Director for Biomedical and Environmental Sciences; Carol Johnson, Richmond's technical assistant; Steve Kaye, director of the Health and Safety Research Division; Amy Harkey, editorial supervisor in the Environmental Sciences Division, and Howard Adler, formerly of the Biology Division and now with Oak Ridge Associated Universities, for their help in planning the contents and soliciting articles for this special issue. We also thank the many contributors for eloquently describing ORNL's exciting research in the life sciences.

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Editorial

Life Sciences Flourish at ORNL — *C. R. Richmond* — 4

Biology Research

Forty Years of Biology Research — *Staff* — 6

GENETICS

Genetics Research in the Biology Division — *L. B. Russell, R. J. Preston* — 12

Chromosome Alterations and Cancer — *R. J. Preston* — 16

Targets for Mutagenesis — *G. A. Sega* — 20

Mouse Models of Human Genetic Disorders — *W. M. Generoso, P. B. Selby* — 24

Mouse Genome Characterization — *E. M. Rinchik, L. B. Russell* — 28

Pursuing Biology's Holy Grail — *C. Krause et al.* — 32

RADIATION BIOLOGY

Radiation-Induced Cancers — *R. J. M. Fry* — 42

Extrapolating Cancer Risk — *J. B. Storer, R. J. M. Fry* — 44

In Vitro–In Vivo Models for Cancer Studies — *R. L. Ullrich* — 47

Probing Mechanisms for Cancer Suppression — *M. Terzagi-Howe* — 49

PROTEIN ENGINEERING

Protein Engineering — *F. C. Hartman* — 52

Altering Human Epidermal Growth Factor — *S. Niyogi, A. Stevens* — 54

DNA Repair—A Recipe for Survival — *S. Mitra* — 57

Can the CO_2 Fixation Enzyme Be Improved? — *F. C. Hartman* — 60

Environmental Sciences

The Evolution of ORNL's Environmental Sciences — *S. I. Auerbach* — 62

ECOLOGICAL SCIENCES

ESD's Educational Impact — *D. E. Reichle, J. L. Trimble* — 72

Ecology: Organisms and the Biosphere — *C. W. Gehrs, R. I. Van Hook* — 80

Biomarkers for Pollutants — *J. F. McCarthy et al.* — 82

Atmosphere–Canopy Exchange in Forests	*G. E. Taylor*	90
Ecosystem Research on Walker Branch Watershed	*J. W. Elwood et al.*	98
Patterns of Landscape Disturbance	*R. H. Gardner*	104

ENERGY AND NATURAL RESOURCES

Problems and Opportunities	*D. S. Shriner, M.P. Farrell*	108
Complexities of Acidic Deposition	*S. B. McLaughlin et al.*	110
Global Carbon Dioxide Studies	*M. P. Farrell*	119
Producing Energy Crops	*J. W. Ranney et al.*	126

COMPLIANCE AND RISK ANALYSIS

Environmental Assessment	*S. G. Hildebrand*	132
Ecology and NEPA: 20 Years and Still a Challenge	*R. M. Reed et al.*	134
Ecological Risk Analysis	*G. W. Suter II et al.*	138
Regulatory Analysis	*F. E. Sharples*	142

EARTH SCIENCES

Geoscience and High-Level Radioactive Waste	*G. K. Jacobs, S. H. Stow*	144
Groundwater Modeling	*L. E. Toran, D. K. Solomon*	148
Water, Rocks, and Waste Disposal	*D. D. Huff, S. H. Stow*	151
Waste Disposal and Environmental Engineering	*E. C. Davis*	154

Health and Safety Research

Health and Safety Research: From Atoms to Humans	*S. V. Kaye*	156

UNDERSTANDING MICROSCOPIC INTERACTIONS

Forty Years of Radiological Physics Research	*G. S. Hurst*	159
Fundamental Interactions in Condensed Matter	*R. A. Ritchie*	162
Ions, Molecules, and Energy	*R. N. Compton, C. E. Klots*	167
Resonance Ionization Spectroscopy	*W. R. Garrett, M. G. Payne*	173
Surface and Submicron Physics	*T. L. Ferrell, E. Arakawa*	179
Radiation and Biological Materials	*H. A. Wright et al.*	182

DOSIMETRY, METABOLISM, AND RISK ASSESSMENT

Ranking Chemical Toxicity	*A. Watson et al.*	186
Interspecies Extrapolation for Risk Assessment	*C. C. Travis*	191

EVALUATING EXPOSURE AND DISEASE

Predicting Radionuclide Transport in the Environment	*S. V. Kaye*	194
Instruments for Monitoring Pollutants	*A. R. Hawthorne et al.*	197
Field Study Motto: Be Prepared	*B. A. Berven, C. A. Little*	201
Information Research and Analysis	*J. T. Ensminger*	206
Nuclear Medicine Research	*F. F. Knapp*	210

Departments

Take a Number	23
R&D Updates	41
Educational Activities	75
User Facilities	141
Awards and Appointments	214

Life Sciences Flourish at ORNL

By Chester R. Richmond

Life sciences research at Oak Ridge National Laboratory is about 45 years old. The program, however, seems young and dynamic because of the many changes over four decades.

As early as 1944, when Monsanto was the federal contractor at Oak Ridge, there was a small biology group headed by Dr. Howard Curtis (who later moved to Brookhaven). The Biology Division really began, however, when Alex Hollaender came to Oak Ridge in 1946. Oak Ridge soon became the mecca for experts in radiation genetics. About 20 researchers who spent part of their careers in the Biology Division were later elected to the National Academy of Sciences; one division member and one consultant are members. The Biology Division continues to change as it becomes more involved in protein engineering and molecular genetics. A source of new excitement is our participation in programs supported by the Department of Energy and National Institutes of Health to map and sequence animal and human genomes.

ORNL has made major contributions to the ecological and environmental sciences. The current Environmental Sciences Division (ESD) grew from the Ecological Sciences Division (formed in 1970), which evolved from the Health Physics Division. Today ESD is one of the world's premier organizations for interdisciplinary environmental research, addressing problems that range in scope from site-specific to global.

In 1977, I established the Health and Safety Research Division (HASRD), merging program elements from several ORNL divisions to focus on human health risk assessment—a niche not filled by other ORNL organizations. The Health Physics Division ceased to exist with the formation of HASRD. During its second decade, HASRD's work in health risk assessment has come of age and is nationally recognized. HASRD's research in nuclear medicine, laser-based detection methods, and pollutant monitoring has contributed significantly to ORNL's success in technology transfer.

Other divisions also contribute to ORNL's life sciences programs. The interactions continue to multiply through interdisciplinary initiatives such as global environmental studies, genome mapping and sequencing, and structural biology. These team efforts are what national labs do best. We are also increasing our collaborations with private industries and academic institutions, making our user facilities and other resources available to academic and industrial colleagues and to science and engineering students and teachers on pre-college, college, and postgraduate levels.

Life sciences research is assuming greater international recognition and importance. Many of the problems facing our planet in this area are global in scope and urgently need solving. Consequently, I believe we will see growth in our Life Sciences programs as we approach and enter the next century.

What are the greatest needs as we approach the last decade of this century? People are our most important resource, so we must do all we can to attract the best employees and keep those we have on our team. We must help employees adapt to the increased priority of safety, equal opportunities for all, and the assurance of quality in every aspect of our work.

Our second most important resource is bricks and mortar. We must secure modern, efficient facilities that meet the needs of our outstanding employees. This is a difficult challenge, but ORNL management is committed to it. I have a vision of a consolidated Life Sciences Complex at ORNL's west end (see drawing on p. 5). This complex would include the current ESD buildings and new buildings for the Biology Division, which has been at the Oak Ridge Y-12 Plant for many years. In fiscal year 1990, a new building for ESD and HASRD scientific and technical information activities will be added to the existing buildings in the complex, and in the 1990s, I hope to see major building additions at ORNL's west end. This expanded complex would house one of the world's best interdisciplinary life sciences organizations.

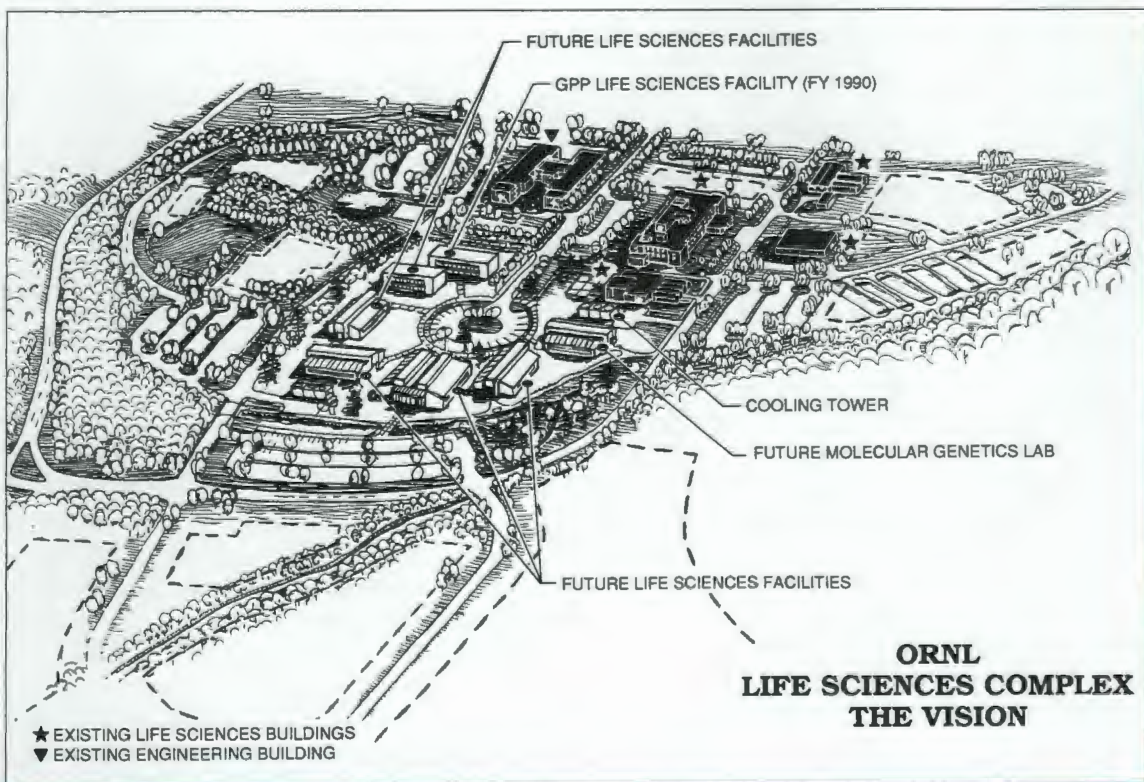
This special issue will provide a glimpse of the past, many examples of our current work, and visions of the future in the life sciences at ORNL. I hope you enjoy reading the articles as much as I enjoy working with everyone who contributes directly or indirectly to the success of these programs. This issue is dedicated to those individuals.

The Vision

Chester R. Richmond, ORNL's Associate Director for Biomedical and Environmental Sciences, has envisioned a consolidated Life Sciences Complex at ORNL's west end to house what Richmond calls "one of the world's best interdisciplinary life sciences organizations." The complex would provide laboratories and office space for employees of the Biology Division, the Environmental Sciences Division (ESD), and the Health and Safety Research Division (HASRD).

The proposed Life Sciences Complex, as depicted here, would include existing ESD buildings (shown in black) and future life sciences facilities. In 1990, the Laboratory is scheduled to receive general-purpose plant project (GPP) construction funds from Martin Marietta Energy Systems to be used for building the Life Sciences Data Analysis Facility. It will house employees of ESD and HASRD who handle data bases and manage scientific and technical information. A recently completed design for a Molecular Genetics Laboratory has been submitted to the Department of Energy as a candidate construction item for fiscal year 1991. If funded, the building would allow some of the Biology Division's personnel now located at the Oak Ridge Y-12 Plant to move to the Life Sciences Complex.

Richmond is committed to this vision of a Life Sciences Complex, emphasizing it is necessary to "secure modern, efficient facilities that meet the needs of our outstanding employees." ornl



ORNL LIFE SCIENCES COMPLEX THE VISION

Forty Years of Biology Research

Richard Kimball (left) and the late Alexander Hollaender seem to be pondering research results. Both served as directors of the Biology Division.

Bone marrow transplants can save the lives of radiation accident victims. Embryos of superior animals can be frozen and implanted in the uterus of inferior animals—a revolution in animal husbandry. Pregnant women are now protected from unnecessary medical X rays and chemical workers from excessive exposure to hazardous chemicals. Consumer action has brought about a reduction of potentially carcinogenic nitrite preservatives in foods. These developments, and others we now take for granted, are in part the result of research done in the Biology Division of Oak Ridge National Laboratory.

Forty years ago, many of these biological advances were only questions that some thought could never be answered: What are the effects of radiation and chemicals on living cells? How is an organism able to exactly duplicate itself, almost cell-for-cell? What makes cancer cells proliferate in uncontrolled growth, and what can be done to stop this process? Could an egg or embryo from one type of animal grow and be born from another type of animal? We don't have all the answers today, of course, but ORNL biologists have made many contributions to solving these mysteries and, in the process, learned much about the genetic process, helped make us safer from radiation and chemicals in the environment, discovered better ways to treat some types of cancer, and developed techniques that will help produce superior strains of plants and animals.

The Biology Division was established as a separate research entity at Oak Ridge National Laboratory in 1946, when Alexander Hollaender, already a distinguished radiation biologist, was hired as director. The new Division initially had two main goals:

- To estimate the genetic and carcinogenic effects of radiation in human populations from studies using biological organisms such as bacteria, fruit flies, and mice; and
- To use ORNL's physical and chemical capabilities, together with the various talents of Biology Division personnel, to advance the developing field of molecular biology.

During the 20 years of Hollaender's leadership, the Biology Division became ORNL's largest division and gained international recognition for its contributions to radiation genetics, biochemistry, radiation carcinogenesis, and molecular biology. This distinction was achieved by a unique investigative style that emphasized a small-scale, interdisciplinary approach to basic research, together with the development of large-scale animal experiments. This approach was first used to determine the effectiveness of radiation in inducing genetic effects and cancer. Former ORNL Director Alvin Weinberg wrote that Hollaender "invented this new style of historical investigation, (which) has forever changed biology."

The new Division soon expanded its programs to include studies of the health effects of environmental chemicals and rapidly gained stature in this area as well. Hollaender also initiated the Biology Division's renowned Gatlinburg Symposia, which

The Biology Division's preeminent studies of mammalian genetics and genetic effects of radiation and chemicals on mammals have had worldwide impact.

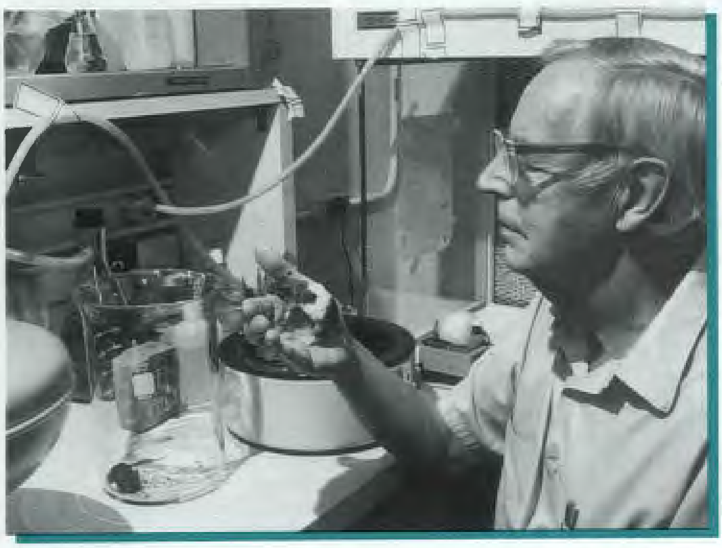
Alexander Hollaender (right), director of ORNL's Biology Division for 20 years, chats with John Wood of the University of Tennessee, Memphis, during one of the series of Gatlinburg Symposia organized by Hollaender.

highlighted the Division's research and provided an opportunity for some of the world's leading scientists to informally exchange biological information. After a short hiatus, this series was reinstated in 1988 with a symposium, organized by Ray Popp and Julian Preston, entitled "Transposable Elements in Mutagenesis and Gene Expression."

Pioneering Studies

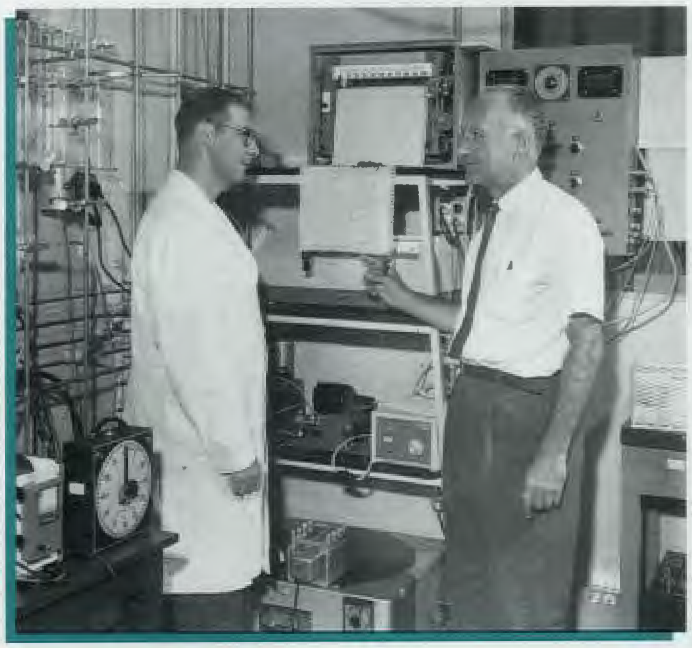
During the 40-year history of the Biology Division, numerous research discoveries have not only advanced our knowledge of fundamental life processes but also found practical applications. This article highlights some of these as a background for the following articles that discuss our current research projects.

The Division's mammalian genetics program, initiated in 1947 by William L. Russell (who retired in 1975) and now headed by his wife, Liane B. Russell (both members of the National Academy of Sciences), has been the world's major source of information concerning the genetic effects of radiation and chemicals on mammals. Findings of this program have guided the development of acceptable limits for human exposure to ionizing radiation. Liane Russell's investigation of radiation effects on mouse embryos at various stages of development have made physicians aware of the potential radiation hazards of X rays to pregnant women. ORNL studies of the mutagenic effects on mice of ethylene oxide, a chemical used to sterilize medical instruments, have led to new regulations about occupational exposure to this chemical.



About 10 years ago, Bill Russell discovered ENU, the most powerful mutagen known.

In parallel with assessing and understanding genetic risks associated with exposure to radiation and chemicals, an analogous program



Waldo Cohn (right) and Mayo Uziel review a chromatographic analysis of modified bases in RNA.

the Department of Energy and the National Institutes of Health to unravel the precise base sequences of entire chromosomes, Dan Lindsley, Rhoda Grell, and Ed Grell in the Biology Division developed a nearly complete functional map of the genome of *Drosophila melanogaster*, using classical genetic crosses and careful examination of phenotypic properties of the fruit fly's offspring.

In the 1940s biological, rather than chemical and physical, methods were used in biology research. Biochemist Waldo Cohn was one of the first to use a chemical approach to a biological problem in 1950, when he, Joe Khym, and C. E. ("Nick") Carter used the technique of ion-exchange chromatography (originally developed for separating fission products) to isolate

investigating carcinogenesis evolved. The large, multidisciplinary effort was once headed by Arthur C. Upton, who left Oak Ridge in 1969 to become head of the National Cancer Institute.

During the 1950s and early 1960s, long before the much-heralded genome initiative launched by

and identify the constituents of nucleic acids, the genetic materials of living organisms. Using this technique, they found that both known nucleic acids (ribonucleic acid, or RNA, and deoxyribonucleic acid, or DNA) have the same general chemical structure. This discovery had a significant

Liane Russell (left) and Clyde Montgomery examine one of the more than 200,000 mice in ORNL's mouse house. Because its male parent had been irradiated, they are checking the mouse for signs of mutations.

worldwide impact on biochemical investigations of these materials.

Much of the early work of the Division was concerned with determining the fundamental structures and functions of these nucleic acids, and several landmark discoveries in the field were made here. In 1956, Larry Astrachan and Elliot Volkin discovered the "messenger" RNA that directs the order in which amino acids assemble to form proteins. In 1964, ORNL biochemists led by David Novelli developed techniques for extracting large quantities of "transfer" RNAs from bacteria, which greatly aided scientists in studying their functions during protein formation.

Elliott Volkin, shown here measuring RNA concentrations, was a co-discoverer of "messenger" RNA.

After it became known that RNA was derived from a DNA template, Oscar Miller was the first to "see" this transcription process two-dimensionally, using electron microscopy; his spectacular pictures of multiple RNA strands peeling off the parent DNA gene like the branches of a Christmas tree were displayed on the cover of *Science* and facilitated his election to the National Academy of Sciences.

More recently, Ada and Don Olins (researchers in ORNL's Biology Division and professors in the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences) used electron microscopy to reveal the basic repeating unit of chromatin (the nucleosome) and to construct a three-dimensional visual image of DNA-to-RNA transcription, which was also published on the cover of *Science*. The Olinses were the first to observe the nucleosome, a subunit of chromatin, which makes up chromosomes (see page 39 for a high-resolution view of the nucleosome). In 1987 they used immunofluorescence to demonstrate in vitro replication, copying a DNA template to synthesize a new DNA strand.

Besides identifying many of the health effects of radiation and various chemicals, Biology Division researchers have also sought to learn how biological systems prevent or recover from damaging effects and how to stimulate these self-protective mechanisms. For example, ORNL biologists studied the "oxygen effect"— chromosomal damage to living cells exposed to radiation in the presence of oxygen—and found several chemicals that protect against the radiation damage by removing oxygen from the cells. They also pioneered experiments showing that cells can repair radiation-induced damage to genetic materials after their radiation exposure ceases.

In the early 1960s, Dick and Jane Setlow came to the Biology Division from Yale University to continue their fundamental studies on cellular mechanisms for repair of ultraviolet radiation damage to DNA. Dick Setlow and Bill Carrier's demonstration (in 1964) of DNA repair in bacteria opened a new field of investigation that has extended to higher organisms, including humans. Deficiencies in such repair mechanisms are an exacerbating factor in several genetic diseases, including predisposition to some cancers. Before leaving ORNL for Brookhaven National Laboratory, where he is Associate Laboratory Director for Biology and Medicine, Dick Setlow was elected to the National Academy of Sciences; in 1988 he received the Fermi Award, the highest honor bestowed upon researchers by DOE.

ORNL scientists were also among the first to develop procedures, such as bone marrow transplants, to reduce the adverse effects of radiation on animals and humans. In 1956, Takashi Makinodan and Charles Congdon of the Biology Division used X rays to destroy the blood-producing cells of experimental mice and replaced them with blood-producing rat bone marrow, showing that the mice could survive and produce rat blood. This work paved the way for the human bone marrow transplants that are used to treat leukemia patients and victims of radiation accidents, such as the one at Chernobyl in 1986.

This photograph of frozen-embryo black mouse pups and the white foster mother who gave birth to them at ORNL appeared on the cover of the October 27, 1972, issue of *Science*.

Studies of cryobiology, led by ORNL's Peter Mazur, have revolutionized animal husbandry through the new technology of embryo preservation and implantation. The first mammalian embryos to be removed, frozen, thawed, and implanted into foster mothers were those of black mice implanted in white mice in a classic 1972 experiment, done by ORNL biologists Peter Mazur and Stanley Leibo and visiting scientist David Whittingham of the United Kingdom. The white foster mother and black pup were featured on the cover of *Science* that year.

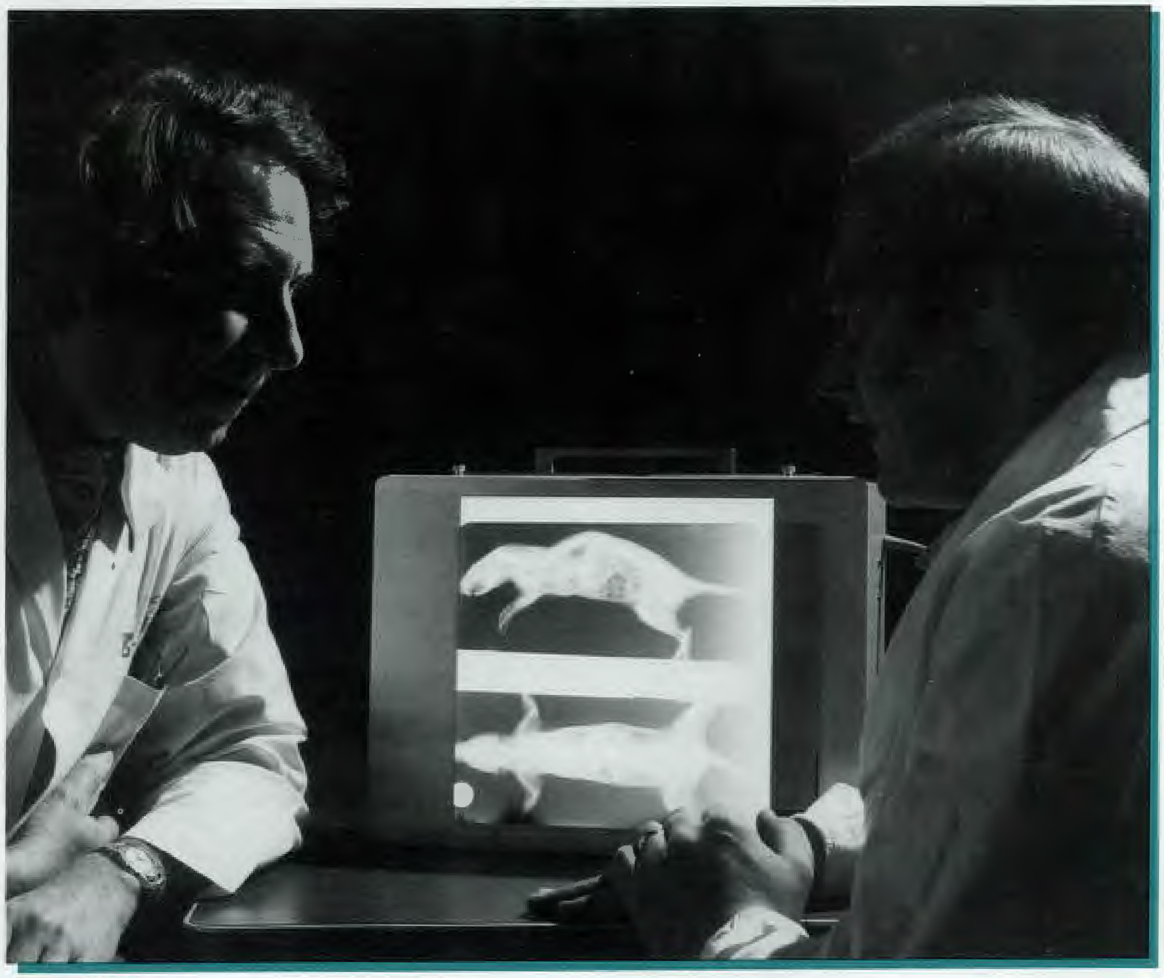
In kinetic studies exposing plants to light-and-dark cycles, William Arnold had uncovered, before coming to Oak Ridge in 1946, some of the basic interacting biochemical mechanisms in photosynthesis. While here he continued his fundamental studies in the water-splitting reaction with a success that was recognized by his election to the National Academy of Sciences.

Peter Mazur pioneered the widely used technique for freezing and storing mammalian embryos for later implantation into foster mothers.

More Achievements

Additional accomplishments of Biology Division scientists include:

- Norman Anderson's development of the zonal centrifuge that is used to purify viruses and vaccines;
- William Lijinsky's demonstration that the nitrites widely used as food preservatives react with amines in foods and drugs to form carcinogenic nitrosamines during digestion in the stomach;
- Sheldon Wolff's characterization of radiation-induced chromosomal breakages;
- David Doherty's discovery of sulfur-containing compounds that partially protect living organisms against radiation damage;
- Fred Bollum's discovery of a DNA-processing enzyme that was instrumental in the early development of cloning techniques and that is used today in leukemia research; and



Willie Lijinsky (left) and Wayne Tayor examine a lung tumor induced in a rat by feeding it an amine and a nitrite.

- Richard Kimball's elucidation of basic mutagenic pathways in unicellular organisms.

The Division Today

Although small by historical standards, the Biology Division today (consisting of more than 150 investigators, guests, and support personnel) remains a vibrant, highly productive research organization with state-of-the-art programs in genetics, carcinogenesis, and molecular biology. Along with biological research in the rest of the world, ORNL's endeavors have been dramatically influenced by the advent of genetic engineering and recombinant DNA technology, which enable investigators to manipulate and alter genes in the laboratory as simply as they do chemical reagents and to transfer genes among different organisms. Within the Biology Division, recombinant DNA technology is used to characterize cancer-causing genes, clarify the mechanisms responsible for regulating gene expression, produce large quantities of scarce proteins for structural studies, design new proteins that do not exist in nature, map mammalian chromosomes, and generate new strains of mice through systematic alteration of genomes, thereby providing strategies for future gene therapy.

Biology Division scientists are active leaders of scientific societies and are members of international editorial advisory boards. Many have received recognition for outstanding research, including membership in the National Academy of Sciences, and several have been recipients of the prestigious Lawrence and Fermi awards by the Department of Energy. The Biology Division's proud history continues. ornl

The *Review* staff acknowledges the assistance of former ORNL Biology Division Director, Richard Kimball, and ORNL Biology Division Director, Fred Hartman, in preparing this article.

Genetics Research in the Biology Division

By Liane B. Russell and R. Julian Preston

Genetic studies are conducted within all sections of the Biology Division, but most take place in two: the Mammalian Genetics Section and the Human Genetics Section. Here we describe the research that has led up to and constitutes the ongoing programs in these sections. Our approach is to explain how classical genetic studies and modern molecular biology techniques have been combined to address important questions pertinent to understanding adverse human health effects.

Mammalian Genetics

The Mammalian Genetics Section was started in late 1947; its original mission was to obtain information on the genetic hazards of radiation. It was obviously desirable to use an experimental organism that was relatively closely related to humans, genetically well characterized, and fast-reproducing. The mouse fits the bill in all respects.

William Russell, who was to head the section for the next 27 years, designed methods for the detection and measurement of various types of genetic effects in mice and developed appropriate stocks of mice. During the 1950s and early 1960s, he led studies that determined the effects of a large array of variables—radiation quality, dose, dose rate, sex, reproductive-cell stage, and age—on mutation rate (frequency of heritable alterations in the offspring of irradiated mice). This body of information provided the basis for projecting and establishing permissible radiation-dose levels for human populations.

In the 1960s, investigators in the section began to study the mutational effects of various chemicals on mice, using several of the methods that had been developed for radiation experiments. Some of this work continues today.

From the beginning, related areas of research also received attention. For example, a clear understanding of the kinetics of germ-cell formation and renewal was obviously necessary for the interpretation of the mutagenesis data. Eugene Oakberg, who retired in 1985, pioneered in this area; his work is still regarded as classic. In the 1950s, the harmful effects of radiation on the developing embryo were systematically identified by Liane Russell. Her results led to a change in medical practice—the use of X rays on potentially pregnant women has been greatly restricted.

Eugene Oakberg, Liane B. Russell, and William L. Russell discuss research strategy in mammalian genetics.

Throughout the section's history, the products of the mutagenesis experiments—mouse strains that carry various mutations and chromosomal rearrangements—served as tools for studies in basic genetics. One outcome of such studies was the discovery of sex-determining mechanisms in mammals; another was finding that one of the two X chromosomes of the mammalian female is genetically inactive.

The work of the present-day Mammalian Genetics and

Major changes in medical X-ray practices, the discovery of sex-determining mechanisms in mammals, and the nation's most outstanding storehouse of mutant gene stocks are some of the legacies of ORNL's 42 years of genetics research.

Development Section has two broad themes. One is investigating the information components needed to understand the mechanisms for the induction of heritable mutational damage and to assess the risk from such damage. These components include

- Detecting heritable mutational damage. Walderico Generoso, for example, is developing methods for detecting chromosome missegregation—a previously neglected genetic endpoint of mutagenesis, and Gary Sega is measuring breakage in sperm DNA, which he finds to be correlated with transmitted chromosome breakage.
- Exploring factors that affect mutational yield, such as the quantity and quality of mutagen binding to molecular targets (see Sega's article on p. 20) or the repair of premutational damage that has been surmised from the various results of experiments with the "supermutagen" *N*-ethyl-*N*-nitrosourea (ENU), discovered by William Russell in 1979.
- Acquiring information not only on mutation rates but on the organismic effects of various mutational lesions. The article on p. 24 describes Paul Selby's findings on mouse skeletal changes that parallel human genetic disorders and Generoso's discovery of congenital anomalies that result from chemical exposures of fertilized mouse eggs.
- Investigating the structural nature of mutations at the chromosome, gene, and molecular levels. Much of the genetic analysis for this purpose has been and is being performed by Liane Russell; the molecular approach is being spearheaded by Eugene Rinchik.

Rick Woychik produces transgenic mice by injecting foreign DNA of known structure into fertilized mouse eggs. Using the inserted DNA as a molecular tag to the genomic region, he can then characterize its structure and function.

A lot of mouse chow is needed for the 200,000 mice in the Biology Division's Mammalian Genetics Animal Facility ("mouse house").

The other broad area of the section's work is the use of altered genes and chromosomes produced in mouse mutagenesis experiments and subsequently maintained as stocks—an unparalleled wealth of genetic tools—for molecular analyses. Particular emphasis is being placed on relating the molecular structure and expression of certain genes or DNA segments to developmental function.

One approach is to investigate chromosomal regions surrounding specific loci (known gene locations) by using large series of radiation-induced, overlapping deletions of genomic segments. These panels of deletions have enabled us to develop functional maps of genomic regions of substantial size (some on the order of 10 to 20 million DNA bases) and are excellent tools both for DNA structural mapping and for fine-structure functional mapping (see Rinchik and Russell's article on p. 28).

A second approach is "insertional mutagenesis" using "transgenic-mouse" technology. Richard Woychik (see photograph on previous page) injects foreign DNA into a nucleus of fertilized mouse eggs and searches for any mutations produced as a result of this disruption of the normal DNA structure. When the inserted DNA of known makeup is used as a molecular tag to the genomic region, both the mutated gene and its normal counterpart can be structurally and functionally characterized and their role(s) in normal and abnormal development can be rigorously assessed.

Human Genetics

Recent advances in the area of molecular genetics have made it possible to take an intimate look at the genotype (genetic constitution) of cells. It is now quite feasible, for example, to characterize a gene mutation at the DNA level by using recombinant DNA cloning techniques and DNA sequencing. Similarly, it is possible to determine (1) the specific types of DNA damage that can be induced by radiation or chemical agents, (2) how these different types of damage can be repaired by enzymes in the cell, and (3) the consequences of misrepair or failure to repair damage on the genotype and phenotype (observed characteristics) of cells, organs, and whole organisms. This work will help clarify the potential for adverse health effects from human exposure to radiation or chemicals.

The new techniques have enabled our geneticists to consider novel approaches to some longstanding problems. In the Human Genetics Section, we are studying the steps from induced DNA damage to mutant phenotype, using mouse models and human cells in culture. Ray and Diana Popp have studied mouse and human hemopathies (blood disorders) for many years. Using molecular techniques and sophisticated cytofluorometric analysis, they have been able to characterize several of these blood disorders—specifically, a mouse ß-thalassemia (hereditary form of anemia) that is a unique model of a corresponding human disorder. We anticipate that this model system can be used to determine the feasibility of gene therapy by employing the normal human gene for reverting the blood disorder. Other model systems are being developed.

Jim Regan, Bill Carrier, and Andy Francis have been studying the defects in DNA repair using cells from persons who show sunlight sensitivity (*Xeroderma pigmentosum*) and exhibit a high frequency of skin cancer. The researchers have identified DNA products resulting from exposure to sunlight that cause damage whose repair is or might be defective. The thymine dimer and its 6-4 photoproduct have been implicated, and we hope that our collaborative studies with Larry Thompson and colleagues at Lawrence Livermore Laboratory will lead to the identification of normal human genes that can correct for the damage caused by these DNA photoproducts.

Some of the research by Julian Preston and his colleagues in the Biology Division's Molecular Mammalian Cytogenetics Group is described in his article on p. 16. In addition to these studies, the group is conducting research (with Sankar Mitra and colleagues) on radon's potential to induce mutations and (with Peggy Terzaghi-Howe and Wen Yang) on radon's ability to transform normal cells to cancerous ones. Rodent cell mutant lines are being used to identify and characterize human DNA repair genes. The use of molecular techniques has made such studies possible.

This brief description of our current genetics-related research serves only to highlight our progress. Major advances are closer now than would have been envisioned only a few years ago. It is exciting to imagine the discoveries that might be included in a similar review two years from now. ornl

Liane B. Russell is head of the Mammalian Genetics and Development Section of the Biology Division. She came to ORNL in 1948 and shortly thereafter received her Ph.D. in zoology and genetics from the University of Chicago. She has received a DOE Distinguished Associate Award and is a Senior Research Fellow of Martin Marietta Energy Systems, Inc. Her other honors include the Mademoiselle Merit Award, the international Roentgen Medal, and election to the Hunter College Hall of Fame and the National Academy of Sciences.

R. Julian Preston's biographical sketch is on p. 19, following his article on "Chromosome Alterations and Cancer."

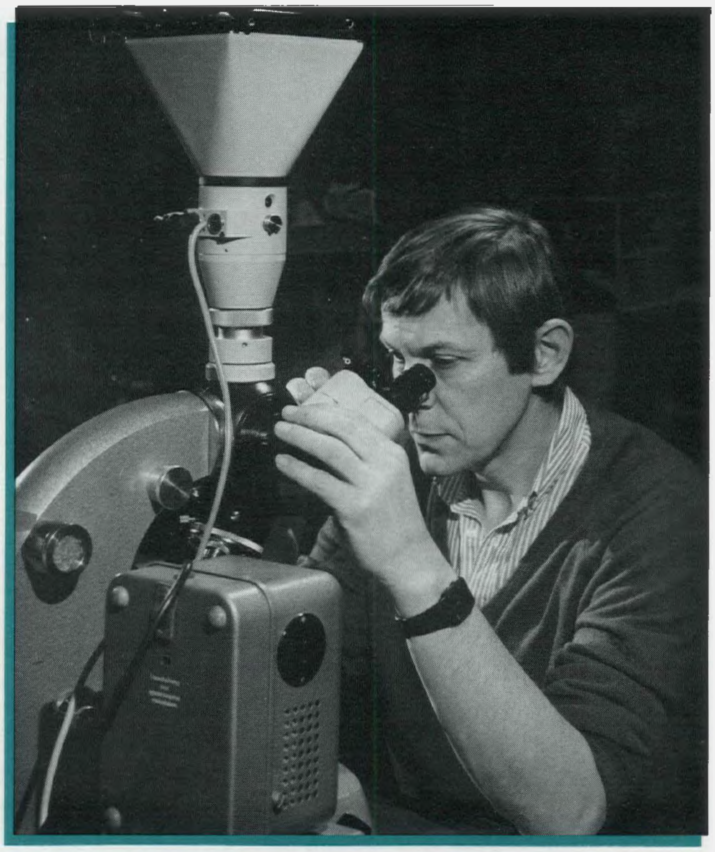
Chromosome Alterations and Cancer

By R. Julian Preston

Chromosome alterations have been observed in all types of tumors studied. In addition, cells of some tumor types, particularly leukemias and lymphomas, show specific chromosome changes. For chronic myeloid leukemia in humans, the specific change is a translocation (exchange of chromosome ends) between chromosome 9 and chromosome 22.

Scientists are trying to determine whether such chromosome changes are involved in the induction, growth, and spread of a tumor or are a secondary effect of the transformation of normal cells to tumor cells. Determining the mechanisms by which specific chromosome changes might be produced or induced in cells is essential to better understanding the etiology of cancers and may lead to improvements in cancer treatment or prevention methods.

We have used a variety of approaches to address the question of how specific chromosome alterations are produced and what role these alterations might have in cell transformation in mouse or rat model systems. Initially, we sought to determine whether spontaneous chromosome alterations or those induced by radiation or chemical agents resulted from errors during the cell's natural repair of DNA damage and/or mistakes during the cell's DNA replication process, when the DNA template is damaged by breaks or altered DNA bases. By using DNA repair/replication inhibitors, we have demonstrated that, for animals exposed to radiation, the majority of chromosome alterations arise from mistakes during the excision repair of damaged DNA bases. But for animals exposed to chemical agents, the majority of the chromosome alterations are produced by errors of replication at the sites of DNA damage. Thus, any induction of specific chromosome alterations by radiation should reflect patterns of DNA repair, whereas nonrandom alterations caused by chemical agents should reflect patterns of DNA replication.



Using a high-powered light microscope, Julian Preston searches for chromosome changes induced by radiation, chemical agents, or restriction enzymes.

A Novel Approach

To gain a better understanding of these phenomena at a molecular level, Richard Winegar (a graduate student at the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences) and I designed a novel approach using restriction enzymes.

Restriction endonucleases are the enzymes used in recombinant DNA techniques, because they can recognize specific sequences of DNA bases—adenine (A), guanine (G), cytosine (C), and thymine (T)—and can make cuts in the DNA within a particular sequence. Different restriction endonucleases have different recognition sequences and cutting points. For example, the enzyme called Msp I recognizes

$$C^{\downarrow}CGG$$
$$GGC_{\uparrow}C$$

and cuts at the site of the arrows, whereas the enzyme Not I recognizes

$$GC^{\downarrow}GGCCGC$$
$$CGCCGG_{\uparrow}CG$$

and cuts at the arrow sites. Msp I will recognize sequences in the genome more frequently, because its recognition sequence length is shorter (4 vs 8 base units).

For our studies, we wanted to determine (1) if restriction enzymes could be introduced into mammalian cells; (2) if they could cut DNA when it was associated with protein in chromatin, the material that makes up chromosomes; and (3) if the DNA cuts made by restriction enzymes could interact to produce chromosome alterations. We developed a technique for introducing the enzymes into cells based on osmolytic shock of pinocytic vesicles. In this method, small membrane-bound vesicles (fluid-filled pouches) are produced from cell membranes by polyethylene glycol. Because the cells used are maintained in sucrose, the vesicles formed will contain both sucrose and the restriction enzyme. A brief hypotonic treatment will preferentially burst the vesicles within the cell rather than the cell itself because of the increased osmotic pressure from the sucrose concentration in the vesicle. We are currently using electroporation (electrically induced "membrane melting") with even more success.

We are encouraged by our preliminary observations that

- all the restriction enzymes we studied can induce chromosome alterations (see figure below), and
- the frequency of alterations is proportional to the calculated total number of cutting sites for a particular enzyme.

This last point is particularly important because our intention is to produce very specific, known alterations, which should be possible using enzymes that have very few recognition sites in a cell. We have very recently used Not I for this purpose. It induces a very low total frequency of chromosome alterations and, significantly, they are restricted to only a few chromosomal locations. These studies tell us something not only about the mechanism by which chromosome alterations are produced but also about possible ways to induce specific changes. We can now begin to study the effects of the specific changes at the cellular level and perhaps determine whether or how certain chromosome changes induce cell transformation.

Chinese hamster chromosomes showing multiple alterations following treatment with a restriction endonuclease.

Chromosome Changes and Mouse Cancers

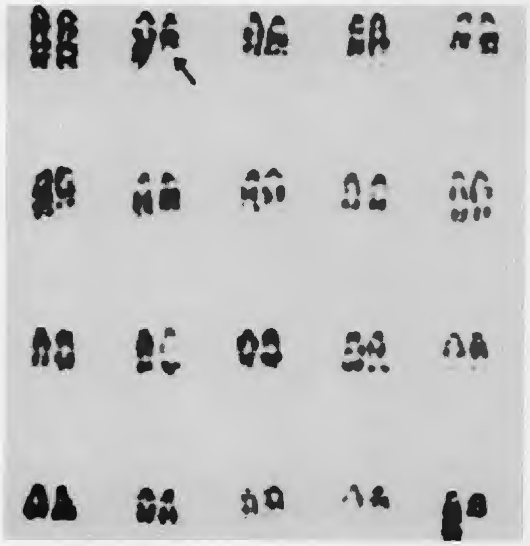
In parallel with these mechanistic in vitro studies, we are using mice to determine whether specific chromosome changes are associated with tumors, particularly myeloid leukemias, sarcomas, and mammary tumors.

In collaboration with Bob Ullrich (now at the University of Texas, Medical Branch), Ti Ho, Henry Luippold, Carolyn Gooch, and I analyzed neutron-induced leukemias. We studied chromosomes taken from the bone marrow and spleens of neutron-irradiated mice, using banding techniques by which each chromosome pair can be distinguished from all other pairs and even small alterations in chromosome structure can be observed. In almost all leukemic animals, there was a deletion of part of chromosome 2 that, regardless of the size of the piece of chromosome lost, always involved a region near a specialized part of the chromosome called the centromere (see figure on this page).

These results suggest that a particular chromosome alteration is involved in the induction of mouse myeloid leukemia. An additional, very surprising, observation supports this suggestion: bone marrow cells analyzed shortly after irradiation to assess radiation-induced chromosome changes showed alterations in chromosome 2 much more frequently than would be expected on a random basis, considering its length. This finding raises the question, Does chromosome 2 have a fragile region?

To help answer this question, Cheryl Bast, Michael Fry, and I have analyzed chromosome changes in plastic-disc-induced mouse sarcomas. The novelty of this system is that, since the sarcoma arises at the site of the disc, the disc can be removed at various times after implantation to check for chromosome alterations or other genetic changes in cells associated with various stages of tumor induction and progression. If the cells from a fully developed solid tumor are analyzed, it is very difficult or impossible to identify any *specific* changes, because so many changes are present (i.e., many extra chromosomes and many structural alterations).

We have not yet identified any specific chromosome changes, but we have obtained good evidence that one step in the development of a sarcoma is the amplification (i.e., production of hundreds of extra copies) of a cellular gene (see figure on the following page). This phenomenon can be observed as a large number of small paired DNA circles (double minutes) in the cells. Molecular techniques can then be used to determine exactly which gene is amplified. Such determinations have shown that, for our sarcomas, none of the genes most frequently amplified in tumor cells (generally, oncogenes or growth factor genes) is being amplified in this case.



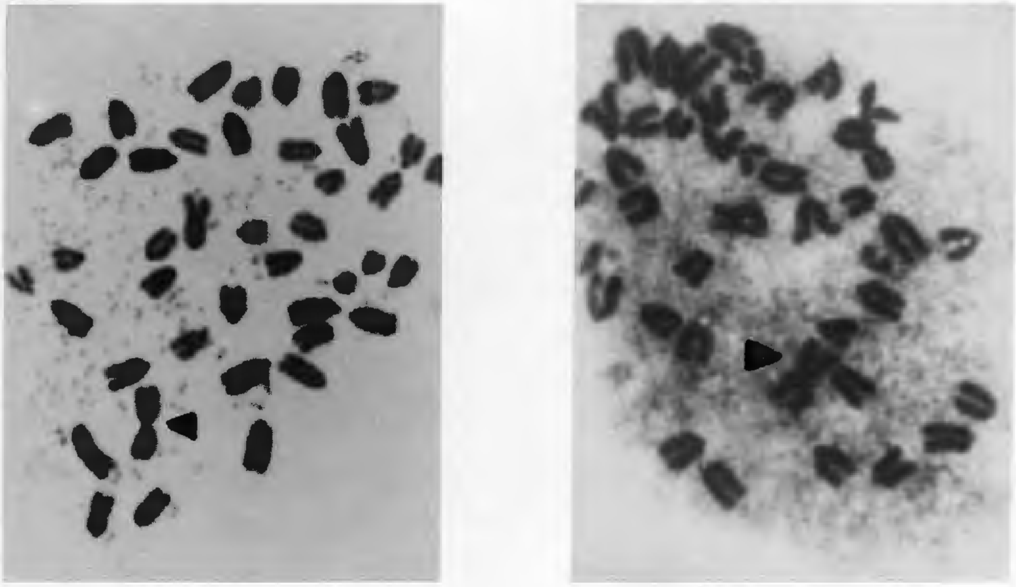
Karyotype of bone marrow cell from mouse having myelogenous leukemia; the arrow indicates a deletion in chromosome 2.

Our very recent experiments have shown that the "multiple drug resistance" gene is amplified—a fascinating result because the animals in which the tumors developed have not been exposed to any drug. This observation has considerable significance for chemotherapy, because tumors might develop that are resistant to some therapy protocols. By understanding this mechanism of drug resistance, the development of improved treatments should be possible.

A collaborative study of the chromosome and gene alterations associated with mammary tumors in mice, done by Susan McKarns, Bob Ullrich, Wen Yang, and myself, has yielded more information on the processes of cell transformation. A specific alteration of chromosome 6 seems to be associated with mammary tumor development. In addition, the amplification of a specific gene (the *myc* oncogene) also occurs in many tumors and appears to be associated with a late step in tumor production, perhaps making the tumor more metastatic (more likely to spread its cells to other parts of the mouse).

These ongoing studies will help us to gain a better understanding of the mechanisms of tumor

a. b.



(a) Mouse mammary epithelial cells showing indicator chromosome (◀) and several double minutes (b) cell from mammary tumor showing indicator chromosome (▶) and enormous numbers of double minutes (small, paired circular DNA fragments).

induction and progression. We are concentrating our attention on chromosome alterations, because sufficient evidence indicates that they are intimately involved in the development of cancers. Our use of restriction endonucleases to produce specific chromosome changes is a novel approach for further investigating the problem. Research in the next few years will lead to a much clearer view of cancer production and, consequently, should help improve both treatment and prevention. ornl

R. Julian Preston, who came to ORNL as a research scientist in 1970, is now head of the Human Genetics Section of ORNL's Biology Division and president of the national Environmental Mutagen Society. A native of London, with a Ph.D. degree in genetics from Reading University in England, he is the recipient of a 1985 Martin Marietta Technical Achievement Award, managing editor of *Mutation Research Letters*, and an adjunct professor at the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences (of which he was formerly associate director).

Gloria Greer, student in the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, cultures human cells and Chinese hamster cells grown in the flask for chromosome research studies.

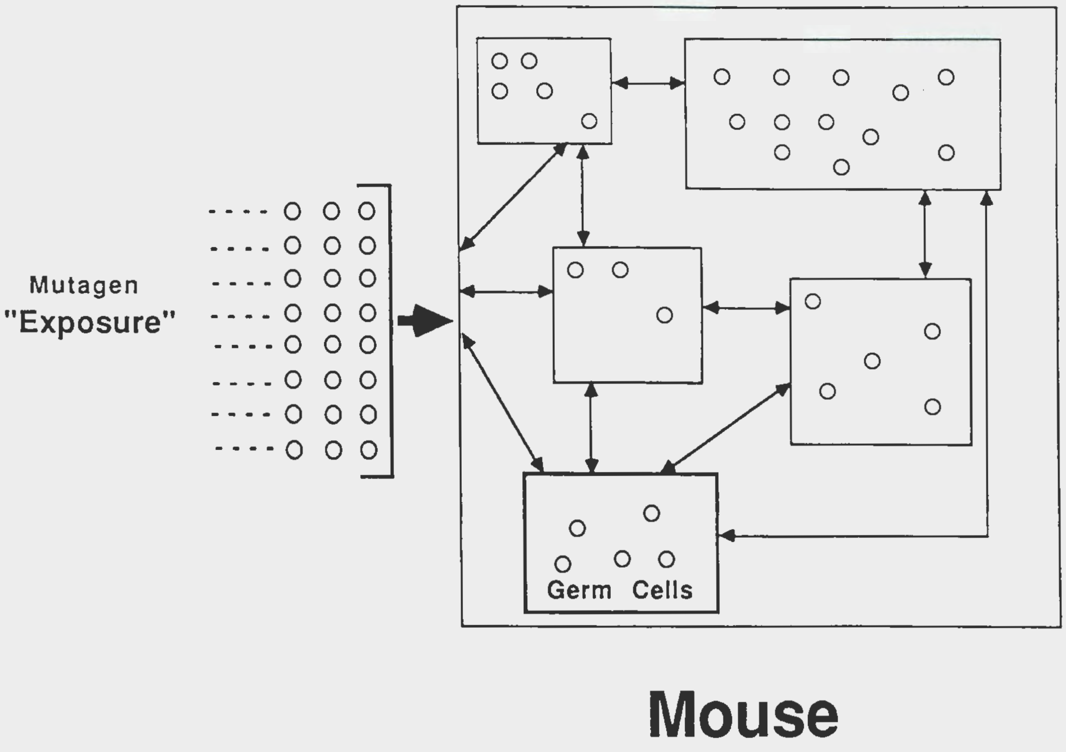
Targets for Mutagenesis

By Gary A. Sega

Environmental chemicals can damage chromosomes in somatic (body) cells and in germ (sex) cells, which pass traits from one generation to the next. If damage to the germ-cell chromosomes is severe enough, dominant-lethal mutations can result, causing the embryos to die before birth. Damage may also involve chromosome breakage and exchange (reciprocal translocation), changes that can be passed on to the next generation, usually causing some sort of detrimental effects. Alternatively, the damage may involve a very small chromosomal region, perhaps even a single deoxynucleotide (a building block of genetic material), giving rise to a single-gene (specific-locus) mutation.

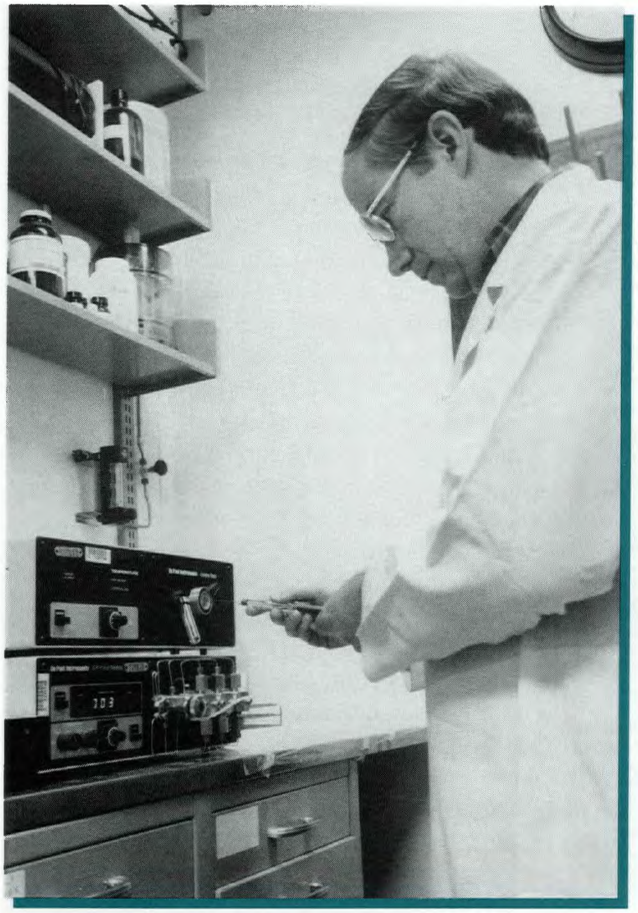
In the development from stem cells to spermatid cells to functional sperm cells (spermatozoa), the male germ cells of all mammals progress through a series of similar stages. For this reason, the male mouse has proved to be an excellent experimental model from which to project the effects of various external agents (radiation or chemicals) on the male germ cells of humans. Many studies have been carried out in mice using a variety of chemical agents that produce differential genetic sensitivity in the various germ-cell stages, depending on the agent being tested.

In studies of the genetic effects of chemical agents in mice, the exposure given to the animals (by injection, inhalation, skin application, etc.) is accurately known. However, we know almost nothing about the molecular dose of the chemical that actually reaches the cells at various stages of development (see figure below). We believe the variability in genetic sensitivity (e.g., chromosome damage) among different germ-cell stages may depend on the availability of the chemical at those stages, the molecular targets of the chemical at the various stages, and the possibility of lesion repair at specific germ-cell stages.



External exposure vs molecular dose: The boxes shown within the "mouse" represent different tissues.

Some chemical mutagens bind to a sperm protein, not DNA, during one stage of a mouse's development.



Gary Sega injects a sample of hydrolyzed DNA into a high-performance liquid chromatography system used to separate various DNA adducts produced by chemical mutagens.

Radiolabeled Mutagens

To study the molecular events that occur within mouse germ cells exposed to chemical mutagens, we use radioactive labeling of chemical agents known to be mutagenic in germ cells. We have studied a number of chemical mutagens in which a radioactive atom, either tritium or carbon-14 , has replaced a corresponding nonradioactive atom (hydrogen or carbon-12) in the molecule. The amount of radioactive chemical taken up by the germ cells can then be traced by techniques such as liquid scintillation counting, which monitors the beta particles (electrons) emitted from the labeled atoms.

These techniques have been used with a number of mutagenic chemicals, including ethylene oxide (EtO), which is widely used in the manufacture of other industrial chemicals and as a sterilant in hospitals, and acrylamide, which is used in paper processing and water treatment. Genetic studies had already shown that these chemicals were especially active in inducing dominant-lethal mutations in late spermatid and early spermatozoal stages, and we wanted to find out why this was so.

After exposing mice to the radioactively labeled mutagens, sperm were recovered from the animals at intervals after exposure and assayed to determine the amount of chemical that was bound (alkylation). For these powerful mutagens, we found a dramatic increase in chemical binding during the late spermatid or early spermatozoal stages (at least an order of magnitude more binding than in other stages). Thus, a correlation exists between increased genetic damage and increased levels of chemical binding to the sensitive stages.

Surprising Observation

To further characterize the molecular nature of the lesions in the germ cells, DNA (deoxyribonucleic acid, the genetic material that comprises the chromosomes found in every cell) was extracted from the germ cells at different stages and assayed for the amount of bound chemical mutagen. To our amazement, the amount of chemical bound to DNA represented only a very small fraction of the total chemical bound to the germ cells. Furthermore, we observed no increase in DNA alkylation in the most sensitive stages (late spermatid and early spermatozoal).

These surprising results forced us to look for alternative molecular targets for mutagenesis within the germ cells. It was already known that in mid- to late-spermatid stages of mammals, the usual chromosomal proteins (histones) are replaced with small, very basic proteins (protamines) whose amino acid composition is more than 50% arginine. In addition, mammalian protamines contain cysteine; in fact, the cross-linking of the cysteine amino acids in protamine (through disulfide-bond formation) gives mammalian spermatozoa their keratin-like properties, similar to some of the properties of fingernails and hair. Because protamine contains nucleophilic sites (e.g., the sulfhydryl group in cysteine), we reasoned that it might be a target for attack by EtO, acrylamide, and other chemicals.

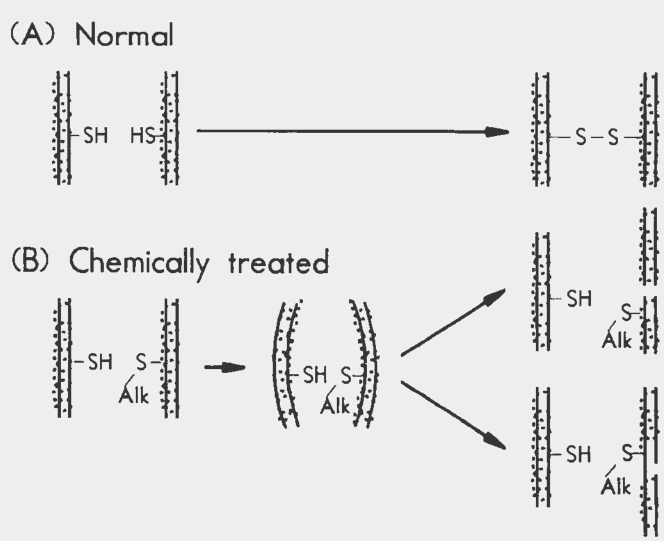
Mutagens Bind to Protamine

When we purified protamine from sperm recovered at various intervals after chemical treatment, we were excited to find that the level of mutagen bound to protamine increased greatly in the most sensitive germ-cell stages (late spermatid and early spermatozoal). The amount of mutagen bound to protamine paralleled precisely the total amount bound to the sperm. In fact, for chemicals that have their greatest effect in late spermatid or early spermatozoal stages (e.g., EtO and acrylamide), we have found that almost all of the binding in the sensitive stages can be attributed to interaction with protamine.

To determine the critical binding locations of mutagens on protamine, we hydrolyzed samples recovered from animals exposed to various agents. Hydrolysis of the protamine (in which chemical bonds are broken by addition of water molecules) breaks it into its constituent amino acids, some of which have sites containing the radioactive adducts formed during sperm exposure to the labeled mutagens. By analyzing the protamine hydrolyzates using an amino acid analyzer and thin-layer chromatography, we have shown that the mutagenic chemicals do, in fact, bind to the sulfhydryl groups in cysteine.

These findings have led us to postulate a model for the binding of certain chemicals to mouse chromatin (chromosomal material) in germ-cell stages that express mutagen sensitivity as chromosome breakage and dominant lethality. In the figure below, the pairs of solid lines in our model represent double-stranded DNA and the broken lines represent the associated germ-cell protamine (which replaces the histones in mid- to late-spermatid stages). The sulfhydryl (-SH) groups are part of the cysteine residues in the protamine, and chemical binding is represented by alkyl (-Alk) groups. In normal nuclear condensation (A), the sulfhydryl groups of cysteine cross-link to form disulfide bridges in the chromatin. However, if chemical binding to a nucleophilic sulfhydryl group occurs before a disulfide bond has formed (B), cross-linking of the sulfhydryl groups cannot take place. This situation could lead to stresses in the chromatin structure that eventually produce either a single- or double-strand DNA break. The end result would be a dominant-lethal mutation or chromosome translocation.

Clearly, not all mutagenic chemicals act by the mechanism described here, and other molecules may be binding targets in other germ-cell stages. However, these observations of the strong binding of some chemicals to sperm protamine in mammals enhance our understanding of mutational processes in male mammalian germ cells. Our results also suggest that failure to detect binding of a specific chemical to DNA does not prove that exposure to that chemical has not occurred. ornl



Model for the chemical alkylation of chromatin in late spermatid and early spermatozoal stages, leading to chromosome breakage and dominant lethality.

Gary Sega, who has a Ph.D. degree in genetics from Louisiana State University, has been a research staff member of the Mammalian Genetics and Development Section of ORNL's Biology Division since 1973. He is a member of the National Research Council's Subcommittee on Safe Drinking Water and DNA Adducts.

Special Properties of Numbers

By V. R. R. Uppuluri

Divisibility by 11

A number is divisible by 11 if, and only if, the sum of its odd-numbered digits (first, third, fifth, etc.) differs by a multiple of 11 from the sum of its even-numbered digits (second, fourth, sixth, etc.). For example, 3080 is divisible by 11, while 3180 is not. Using this principle, it can be determined that 31,680 nine-digit numbers are divisible by 11, no digit is equal to zero, and no two digits in these numbers are alike.

Some Special Numbers

Some numbers have special or unique properties. For example, 81 has the special property of being equal to the square of the sum of its two digits:

$$81 = (8 + 1)^2 = 9^2 .$$

This is the only two-digit number having this property.

Three four-digit numbers have the special property of being equal to the square of the sum of the number obtained by splitting the number into two halves and adding the two parts. These are:

$$2025 = (20 + 25)^2 = 45^2,$$

$$3025 = (30 + 25)^2 = 55^2,$$

and

$$9801 = (98 + 01)^2 = 99^2.$$

The only two six-digit numbers having this property are:

$$494209 = (494 + 209)^2 = 703^2$$

and

$$998001 = (998 + 001)^2 = 999^2.$$

Mouse Models of Human Genetic Disorders

By Walderico M. Generoso and Paul B. Selby

Mutagenesis research in mice has two major objectives: evaluating genetic risk and understanding basic mammalian biology. The ultimate goal is to apply these findings to improving human health. Mouse mutagenesis research contributes to the understanding of developmental anomalies by studying the nature and effects of mutations and the mechanisms of mutation induction and transmission.

Mutation Studies

In both mice and humans, mutations often cause developmental anomalies, such as a defective limb. Understanding the series of processes by which any given mutation produces a congenital anomaly is a major undertaking and one of the most difficult challenges in mammalian biology. These processes are complex, and studying them in humans is generally not feasible. Analogous mutants in laboratory mammals provide our best research opportunity. Mouse mutants, in particular, are valuable because of a relatively rich genetic linkage map and because we have extensive biochemical and molecular genetics information on mice.

Four of the developmental mutants in mice that we and our collaborators are currently investigating are classified as chromosome rearrangements (transpositions of chromosome parts), and one is classified as a dominant gene mutation (or "small deficiency").

One of these mutants has an appendicular abnormality in which the hind legs are splayed and toes on all legs are stubby and sometimes fused. This anomaly is expressed in the homozygous state (inheritance from both parents) of a rearrangement involving two chromosomes. The chromosome rearrangement "unmasks" the expression of the recessive *ld* (limb defect) gene. The molecular mechanism by which this occurs is of particular interest, because the human genome carries a considerable load of deleterious recessive genes that are masked in the heterozygous (inheritance from one of the parents) state.

Paul Selby watchfully examines two mice, one of which (left) has a misshapen head caused by a mutation.

Several recessive limb-deformity mutations are known in humans. Our limb-defect rearrangement mutant stock is currently being investigated for possible analogy with a human mutation.

In the past, it was generally believed that, except for reduced fertility, heritable reciprocal chromosome translocations had no harmful effects in heterozygotes. An increasing number of exceptions have now been demonstrated both in mice and in humans, raising the possibilities that (1) certain chromosome rearrangements affect gene expression and that (2) some human genetic disorders assumed to be the result of single-gene mutations may instead be linked to chromosome rearrangements that have dominant effects.

The mouse diver mutant carries a reciprocal chromosome translocation and exhibits subtle neurological symptoms, as well as an inability to swim. Serendipitously, one of the breakpoints of the rearrangement is closely linked to the *ld* locus, whose molecular structure is being studied by Richard Woychik of the Biology Division. The diver mutant promises to be a valuable model for studies of both the biochemical mechanisms responsible for neurological manifestations and the molecular alterations at the chromosomal breakpoints that lead to dominant expression of the rearrangement.

Most individuals having a heterozygous reciprocal translocation do not, themselves, exhibit an abnormality other than reduced fertility resulting from early embryonic death. In most cases of chromosomal imbalance, the embryonic death is so early as to go unnoticed and have little or no medical importance. However, among humans, certain individuals having chromosomal imbalance survive and must cope with their congenital malformations, posing a health care problem. Our systematic study has revealed that translocations producing these congenital defects are far more frequent than previously thought. Consequently, we need to determine the cytogenetic nature of these rearrangements and the meiotic processes that are responsible.

Morphogenesis Research

In addition to being the subjects of fundamental studies, these chromosome translocation stocks are a dependable source of specific malformations. Collaborators from other institutions use them as models for studies of morphogenesis (the formation and differentiation of cells making up tissues and organs) that could not be carried out using human subjects. Joe C. Rutledge, of the University of Washington School of Medicine in

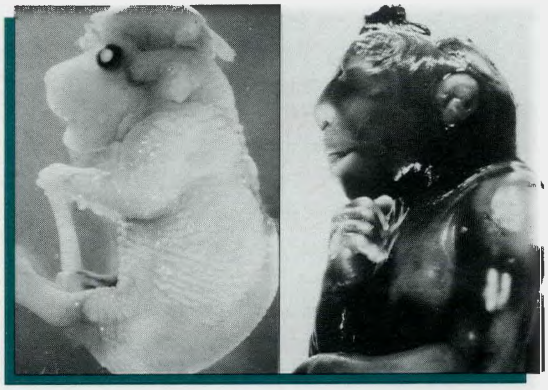
K. T. Cain compares a limb deformity mutant with a normal mouse.

Seattle, is collaborating with us on an animal study of an exposed-brain defect that is also common in humans (see figure on this page). We are trying to determine whether the malformation arises directly from faulty bone structure in the skull or indirectly from a defective neural tube.

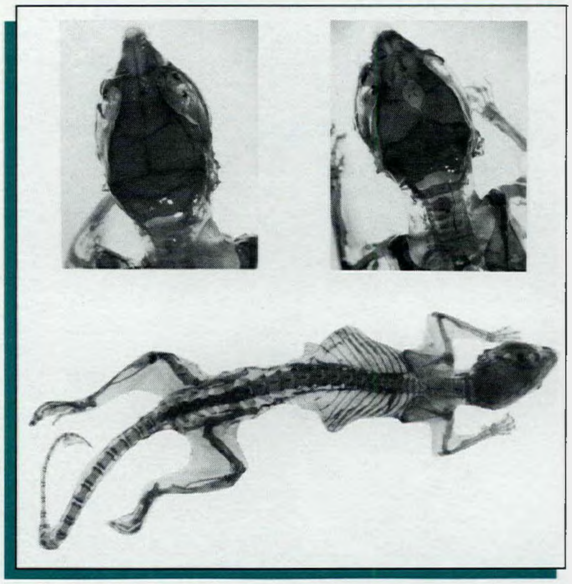
Another translocation defect in mice that we are studying is clefting of the palate that extends externally along the snout. Similar facial clefting occurs in humans (see figures on p. 27). We are collaborating with Kathy Sulik, of the University of North Carolina Medical School at Chapel Hill, to study the morphogenesis of this defect, and we are comparing our rearrangement stock with a transgenic stock that has similar facial clefting.

Mice and humans have basically similar skeletons. Furthermore, many mutagen-induced skeletal mutants in mice have malformations analagous to some seen in humans. In both species, the inheritance of the disorders is often irregular. That is, sometimes an individual carrying the mutation appears normal. When the malformation is present, the severity often varies greatly from one individual to another, and the reasons for this variation are not understood.

Another mouse mutant being used in studies of abnormal development shows a condition called cleidocranial dysplasia, which has an exact human homolog (see figure below).This mutation, which was found by Paul Selby, is either a gene mutation or a "small deficiency." In both species, some or all mutants show incomplete development of the clavicles (collar bones) and many other effects. People with the disorder can often almost touch their shoulders together and have many of the malformations seen in mice, which include a characteristic hole in the skull, extra subdivisions of skull bones, poorly developed nasal bones, and abnormalities of the shoulder blades, hip bones, and vertebrae.



Both the mouse fetus (left) and human fetus have the exposed brain syndrome, or neural tube defect.

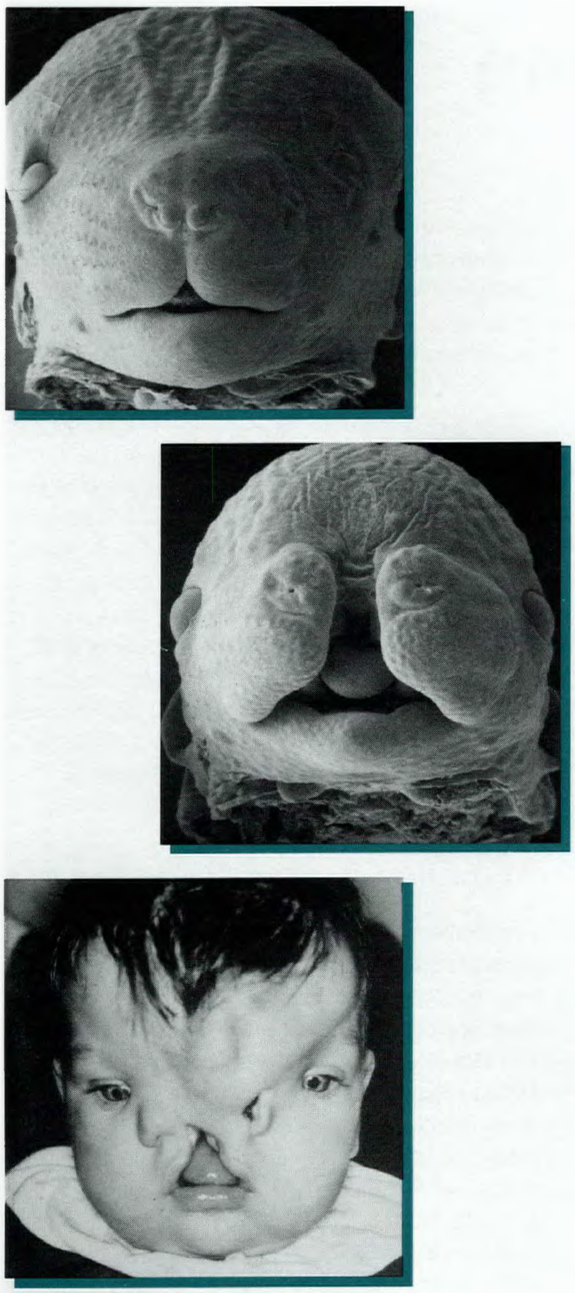


Mouse skeleton with insets showing normal skull (left) and skull with cleidocranial dysplasia (note hole in center skull where bone plates are incomplete).

Currently, there is a surge of interest in studying these mouse mutants associated with developmental defects. Newly induced mutations that have dominant effects are most important in terms of risk to the next generation. Chromosomal rearrangement mutants are particularly valuable, because the breakpoints mark the genetic locations of the defects and provide a convenient starting point for molecular studies. Our systematic collaborative approach that combines genetic, cytogenetic, anatomical, and molecular studies should make valuable contributions toward understanding the complex pathway involved in abnormal development.

Experimental Embryopathy

There are three generally recognized methods for inducing congenital anomalies in laboratory mammals. Conventional teratogenesis studies (i.e., studies involving the treatment of pregnant females) are known to produce high frequencies of fetal malformations, particularly when the female exposure to radiation or chemical agents occurs during the period when their embryos are forming major organs (between days 6 and 13 of gestation in mice). Second, exposures of parents to certain mutagens before mating are also known to cause congenital anomalies. The third common method for producing developmental mutants for basic



The mouse at left has normal facial structure. The mouse at center and human at bottom display the same type of facial developmental abnormality, known as frontonasal dysplasia.

studies is the integration of transgenes (exogenous, or foreign DNA sequences) into the mouse genome.

In our research, we developed yet another method when we discovered that high frequencies of varied fetal malformations were induced by allowing the female mice to inhale high concentrations of ethylene oxide (EtO) near the time of egg fertilization or during early stages of zygote development. The fetal malformations produced are similar to common sporadic human defects for which the etiology is usually unknown. Ethyl methanesulfonate (EMS), another mutagen, produces similar effects when administered intraperitoneally. The EtO findings are particularly important, because this industrial chemical is used extensively as a sterilizer in hospitals.

In studying the possible mechanisms for this embryopathic phenomenon, we conducted a reciprocal zygote-transfer experiment, which showed that the effects were not mediated by the maternal environment. We also did a first-cleavage cytogenetic study, which found no obvious chromosome aberrations. Thus, although the evidence suggests a genetic basis for the malformations, the mutagenic damage is of a type not yet identified in mammalian mutagenesis. Robert K. Fujimura, a member of the Biology Division group doing these studies, is currently investigating the possible molecular mechanisms.

Our findings are of major interest in evaluating the safety of these chemicals and in studying the etiology of congenital abnormalities. They raise questions regarding the vulnerability of human zygotes when the mother is exposed to such environmental chemicals. Our research on the mechanisms involved may lead to greater understanding of the human congenital malformations for which the etiology is still unknown. ornl

Walderico M. Generoso, a senior scientist in ORNL's Biology Division, is a native of the Philippines and holds a Ph.D. degree in genetics from the University of Missouri. He has edited three books on mutagenesis, has participated in studies for the National Academy of Sciences, National Research Council, and the Environmental Protection Agency, and currently serves on the editorial boards of three biology journals.

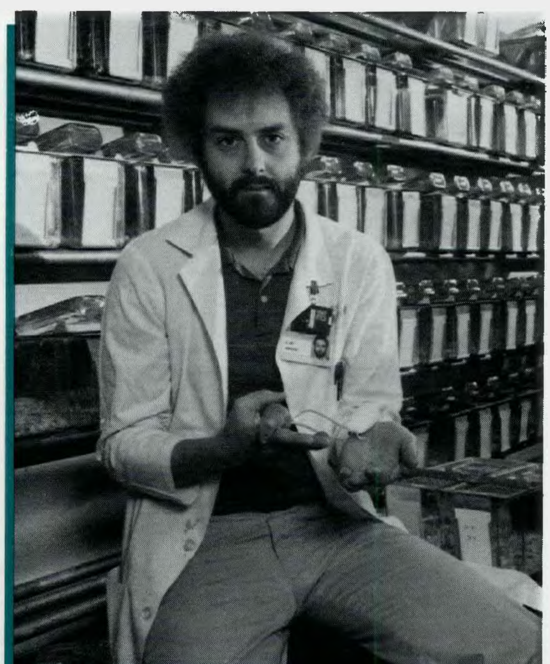
Paul B. Selby, a senior scientist in ORNL's Biology Division, is a recipient of DOE's prestigious E. O. Lawrence Memorial Award. Selby, who holds a Ph.D. degree in biomedical sciences from the University of Tennessee, is a member of the U.S. delegation to the United Nations Scientific Committee on the Effects of Atomic Radiation and has served on committees appointed by the National Research Council and the National Council on Radiation Protection and Measurements.

Mouse Genome Characterization

By Eugene M. Rinchik and Liane B. Russell

The genome—the sum of all genetic information contained within each cell—plays a critical role in the life cycle of all living organisms. The human and other mammalian genomes are extremely complex, both in their organization and structure at the DNA level and in the functions and developmental processes that they must specify and regulate throughout life. Nonetheless, the genomes of all mammals (including man) have much in common, making it possible to use nonhuman mammals as model systems for basic genome research.

Mice are desirable experimental substitutes, or models, for the study of normal and abnormal human developmental processes (including processes often associated with certain genetic abnormalities and diseases). They are easily managed experimentally and are similar to humans in many aspects of anatomy, biochemistry, and physiology. In the Biology Division, we are currently applying strategies of molecular developmental genetics to the mouse model system to obtain basic information about the composition and structure of regions of the mouse genome. Our approach emphasizes the study of mutations, which often result in aberrant developmental processes, to learn more about the corresponding normal life processes.



E. M. Rinchik investigates the composition and structure of genetic regions of mutant mice to better understand the function of specific genes in normal developmental processes.

Heritable mutations can often define and clarify the genetic control of individual developmental pathways by creating abnormal phenotypes (new variations of an organism resulting from changes in its basic genetic information, usually called its genotype) that can then be compared with those specified by the normal genome. Consequently, our research strategies include the detection, molecular and functional characterization, and experimental exploitation of germline (heritable) mutations of the mouse. These strategies are yielding valuable insights into the normal composition and function of segments of the mammalian genome.

Mutations Preserved

Extensive mutation-rate experiments, designed to test for the potentially adverse genetic effects of various types of radiation and chemical mutagens, have been carried out in the Mammalian Genetics and Development Section of ORNL's Biology Division for nearly four decades. Most of this work has concentrated on deriving quantitative measures of mutation rates. However, many of the mutations obtained in these experiments have been preserved through propagation in breeding stocks or through storage as frozen eight-cell embryos and constitute a type of organic "bank" of genetic lesions and their associated physical effects.

A wide variety of mutagenic effects is stored in these stocks, ranging from relatively "simple" changes in coat color or ear length to extremely severe effects, such as skeletal or neuromuscular abnormalities and stage-specific embryonic, fetal, newborn, or juvenile death. Mutations manifesting even the most "minor" of effects can identify genes at loci (locations) within the genome that contribute to a specific process, serving as genetic "markers" to researchers studying that process. For example, mice carrying the *se* (short-ear) mutation in chromosome 9 also exhibit associated abnormalities in the entire cartilage skeleton, as well as the obvious defect in ear length.

In addition to measuring mutation rates and characterizing and preserving mutant stocks, the Biology Division's Mammalian Genetics and Development Program continues to generate new mutations, which either define new loci within the genome (hence, new identifications of corresponding biologic processes) or provide variants that differ in severity of effect or degree of phenotypic complexity at already-defined genomic loci. We have been able to develop and use specific breeding protocols that preserve even highly detrimental mutations indefinitely, making them available for future genetic studies.

Chromosomal Deletion Mutations

Radiation-induced lethal mutations that have occurred at several specific regions within the mouse genome comprise an important subset of these heritable mouse abnormalities. The mutations are termed "lethal" because the animals that carry them die prematurely at various life-cycle stages (depending on the mutation), unless the wild-type (normal) counterpart of the gene is also present. Our extensive genetic analyses of mutations of this type have revealed that most are "multilocus" deletions of entire genomic regions and that many genes (hence, functions) are missing. For example, a certain radiation-induced mutation at the *c* (albino) locus and one at the *p* (pink-eyed dilution) locus, both in chromosome 7, together delete ~15% of the genetic material normally contained within that chromosome, corresponding to ~0.6% of the entire genome. Our genetic analyses of these mutations have provided evidence for many previously undescribed genes that control specific aspects of normal embryonic, fetal, and neonatal development; fertility; metabolism; juvenile fitness; neurological well-being; and pigment-cell structure and biochemistry.

We are currently conducting a detailed molecular and functional analysis of the fraction of the mouse genome associated with this type of radiation-induced deletion mutations. New dimensions have been added to our studies through the constant evolution and improvement of molecular biology techniques. We are now better able to identify and biochemically characterize single genes and to correlate our genetic analyses of basic DNA defects (i.e., mutations) with the organic consequences of such defects.

Our experience has repeatedly shown that deletion mutations are ideal "reagents" for beginning the molecular analysis of a specific chromosomal region and its associated genes. As one example, we are currently isolating DNA clones from libraries enriched for chromosome-7 regions or subregions to identify the DNA sequences that are missing in the *c* and *p* deletions already discussed. New techniques such as fluorescence-activated chromosome sorting and chromosomal microdissection and microcloning (in collaboration with T. Magnuson at Case Western Reserve University) are being used to construct these *c*- and *p*-region–enriched libraries. The direct mapping of DNA clones to region-specific deletion mutations, along with other new genome-analysis techniques, allows us to bypass the more laborious standard genetic linkage experiments. Our "deletion-mapped" DNA clones can then be used as molecular access points to begin identifying and characterizing the individual genes and functions associated by genetic analysis with the multigene-deletion mutations.

Detecting Deletions in Chromosomes

The molecular detection of chromosome deletions employs a sequence of sensitive biochemical manipulations. In the "Southern blotting" method, DNA is first chemically isolated from tissue of a laboratory mouse and fragmented by digestion with an enzyme, restriction endonuclease. The digestion process breaks the DNA at specific sites, yielding a population of double-stranded DNA fragments of various lengths. The fragments are then incorporated in an agarose gel, separated according to size by an electrophoresis process, transferred and bound to a nylon support membrane, made single-stranded, and "hybridized" to a specific radioactively labeled DNA fragment "clone."

The labeled DNA clone bonds to the corresponding single-stranded genetic fragment of mouse DNA under investigation, and the excess (unbonded) radioactive DNA fragments are removed by washing. The remaining bonded radioactive genetic sequence produces horizontal band images on X-ray film like those shown in the figure below. Each vertical "lane" of this X-ray image represents DNA fragments from one test animal. The upper, heavier bands in each lane are produced by a specific chromosome-7 gene sequence, and the lower, paler bands represent a chromosome-9 sequence used as a control. If the mouse DNA gene sequence that corresponds to the radioactive "clone" is missing (deleted), no band image appears in that position. Researchers then compare the X-ray images produced by both "wild-type" (normal) and mutant mouse strains to identify the gene sequences and locations associated with particular abnormalities.

Mutant mouse strains missing a specific cloned DNA sequence are identified by the five empty spaces in the top row of this X-ray image. The band images shown here are produced by radioactively labeled DNA fragments from 11 laboratory animals.

The study that produced the images shown here involved DNA fragments from 11 mice. Lane 1 (extreme left) is derived from a normal mouse and lanes 2 through 11 are from mutants. The mutant strains represented by lanes 5 through 9 are missing this particular chromosome-7 gene sequence (missing bars in the top row). None of the mice in this study have missing chromosome-9 genes (bottom row).

Interestingly, previous genetic analyses have demonstrated that male mice with this particular chromosome-7 DNA deletion are albino, runted, and sterile; female mice with this deletion are albino, runted, and, although able to conceive, are unable to maintain a normal pregnancy. By this type of Southern blot analysis, we have been able to determine that this particular cloned fragment of chromosome-7 DNA (our probe) is derived from the chromosomal subregion associated with these abnormalities.

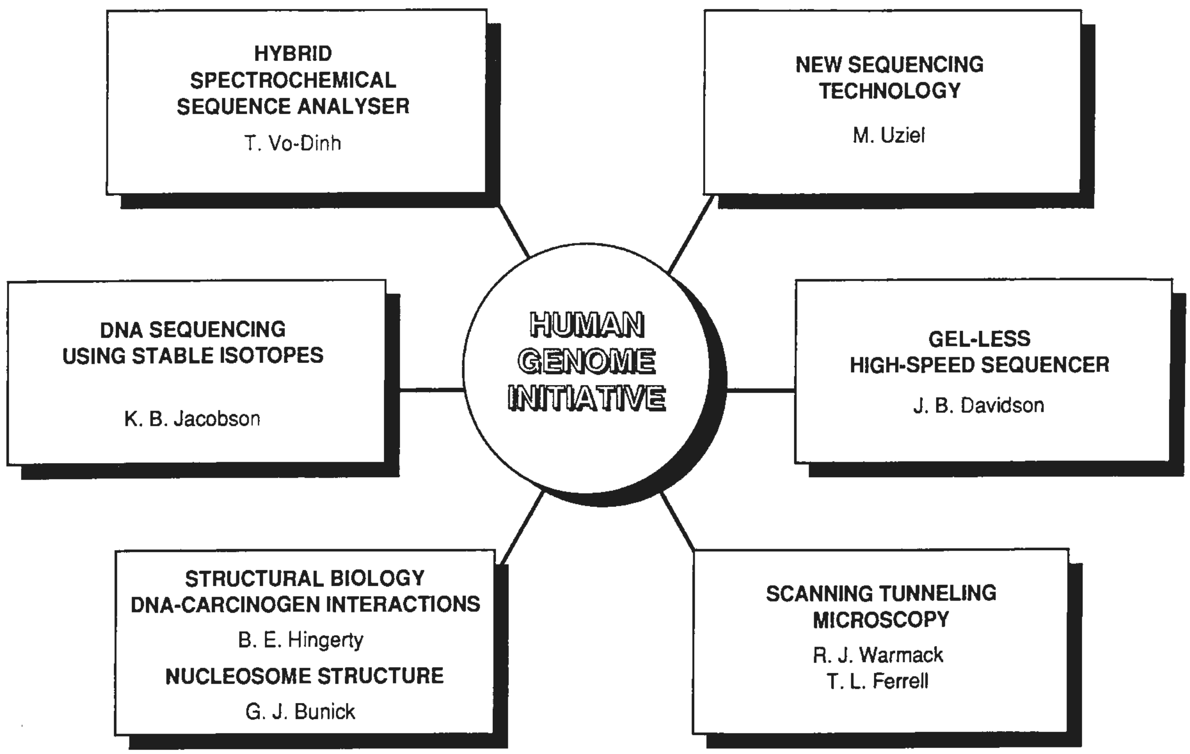
While the radiation-induced deletion mutations are excellent tools for in-depth molecular study of regions of the mammalian genome, our researchers also use them in saturation-mutagenesis experiments. The long deletions are again exploited as special "genetic reagents." In this case, they enable us to refine the genetic and functional mapping of chromosomal regions even further, by detecting newly induced, single-gene mutations (in contrast to the previously discussed multi-gene deletion mutations) that result in specific, characteristic, mutant phenotypes. As our molecular maps become more detailed and more complex, our bank of individual-gene mutations that specify particular mutant phenotypes will comprise the next generation of genetic reagents to be used for the ultimate correlation of DNA composition with function.

We believe our integrated molecular and genetic analyses of mouse-genome deletion mutations will contribute significantly both to the analysis of other complex genomes and to basic research in human genetics and biology. Studies of some of the mouse mutations (and their corresponding abnormal DNA sequences) will be useful models for investigating human genetic disorders and abnormalities and will aid researchers in augmenting the already-recognized homologies in genome structure and function that exist between mice and humans.

Our molecular and functional characterizations of regions of the mouse genome also have the potential for ascribing functions to human genomic sequences that might otherwise remain unknown or be characterized at a less-specific level. Geneticists have only begun to define and understand the biological complexities of the mammalian genome, and we anticipate exciting progress in this area. ornl

E. M. (Gene) Rinchik, a researcher in the Mammalian Genetics and Development Section of the Biology Division, came to ORNL in 1987. He has a Ph.D. degree in genetics from Duke University.

Liane B. Russell's biographical sketch is given on p. 15.



This schematic shows ORNL work related to characterizing the human genome. This work is supported by the ORNL Director's R & D Fund. For an overview, see the following article.

Pursuing Biology's Holy Grail

Compiled by Carolyn Krause

One of the most recent and exciting U.S. science initiatives is the Human Genome Project. The human genome, which has been called the "holy grail" of biology, is the complete set of genes responsible for the development and functioning of a human being. The goal of the national project is to determine the chromosomal locations and molecular composition of the 50,000 to 100,000 genes and regulatory elements that make the human genome. The main justification for the $3 billion project is that knowledge of gene location and structure should greatly improve the diagnosis and treatment of most human diseases, ranging from single-gene disorders such as cystic fibrosis to complex illnesses such as cancer.

The Human Genome Project is formally directed by the National Institutes of Health, with input and funding from the Department of Energy. Two DOE national laboratories—Lawrence Berkeley and Los Alamos—have been designated human genome research centers.

However, several other national labs, including ORNL, are conducting research related to characterizing the human genome. Six ORNL researchers doing genome-related research—Gerry Bunick of the Solid State Division, Jack Davidson of the Instrumentation and Controls Division, Brian Hingerty of the Health and Safety Research Division, Bruce Jacobson of the Biology Division, Mayo Uziel, and Tuan Vo-Dinh, both of the Health and Safety Research Division—are supported in their work by the ORNL Director's Research and Development Fund. In addition to this work related to "sequencing technologies," important research on "mapping" as well as sequencing the mouse genome is being conducted by Liane B. Russell and E. M. (Gene) Rinchik of the Biology Division (see article on p. 28). The scanning tunneling microscope in the Health and Safety Research Division, which recently imaged the tobacco mosaic virus with a resolution of 0.1 nm, may also be useful for imaging and sequencing DNA bases.

Mapping and Sequencing

Human genome research focuses on mapping and sequencing the genome. Two kinds of maps are being developed: "physical maps," which show the locations of specific genes on specific chromosomes, and "genetic linkage maps," which associate specific genes or parts of chromosomes with certain inherited functions or disorders (e.g., color blindness or manic depression). Chromosomal locations of more than 1215 genes, including those responsible for 20 common genetic diseases, have already been determined.

Sequencing involves determining the order of nitrogenous bases attached to DNA's phosphate backbone. The order of these bases can determine the order of amino acids in more than 50,000 different proteins that carry out life's processes. The total human cellular DNA is a linear sequence of roughly 3 billion pairs of bases packaged into 23 pairs of chromosomes (including a pair of sex chromosomes). There are four different DNA bases—adenine (A), thymine (T), cytosine (C), and guanine (G), which may be arranged in many different sequences. A fifth base—5-methylcytosine (m^5C)—usually acts the same as regular cytosine in double-stranded DNA, but it also appears to play a role in controlling gene expression. The DNA molecule consists of two strands that are connected by hydrogen bonding of the bases across the strands to give the familiar double helix. Since base A generally pairs with T (TA or AT), and C with G (CG or GC), the sequence of bases in one strand will determine the sequence in the other strand, thus allowing for the faithful reproduction of the DNA molecule. Each gene has a particular sequence of bases, and determining this for each gene is called sequencing.

In his article, "The Human Genome Project," published in the September–October 1988 issue of *American Scientist*, Charles DeLisi, a former DOE official who started the Human Genome Initiative in DOE, writes, "Humans differ from their closest evolutionary neighbors by approximately one base in 100; unrelated humans differ from one another

ORNL research could lead to faster characterization of the human genome.

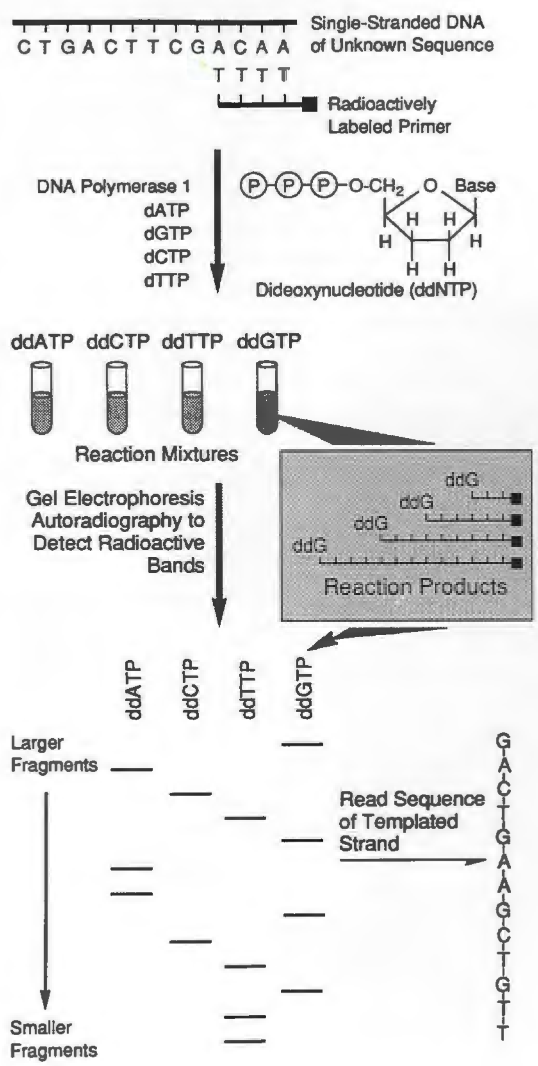
by about one base in 1000. These differences in the human population, when they occur at key locations, can have a pronounced effect on predisposition and resistance to particular diseases."

Determining the base-pair by base-pair composition of genes is slow, tedious work, but sequencing activities are under way at a number of laboratories. "At present," states DeLisi, "relatively little is understood about the human genome, although progress has been rapid. If the number of bases sequenced is a rough correlate of what is understood, we are only about 1/1000th of the way there, with over 20 million bases now in the computerized gene library at Los Alamos National Laboratory. At the present rate, the genome will be sequenced and understood by the year 2700." Currently, individual groups using the most advanced methods can sequence 10,000 to 15,000 bases per day; DOE's goal is to support development of instrumentation and procedures that will raise the U. S. total to a million bases sequenced per day. A number of scientists, including ORNL researchers, are working on ways to increase the rate of sequencing bases by improving the partially automated techniques currently used or by developing an entirely different technique.

Sequencing Technologies

Two manual sequencing processes developed in the 1970s are still in use today. They are a chemical degradation method, developed in 1977 by Alan Maxam and Walter Gilbert of Harvard University, and a chain-terminating method, developed in 1975 by Frederick Sanger and Alan Coulson at the Medical Research Council in Cambridge, England. Both methods use gel electrophoresis in which DNA fragments are separated according to size in a polyacrylamide gel under the influence of an electric field (producing a ladder of fragments, each one differing from its neighbor by a single base). The techniques are similar in that the sequencing reactions produce four sets of radioactively labeled DNA fragments that are each one nucleotide longer than its neighbor. The two methods differ in the ways that the fragments are produced.

In the Maxam-Gilbert technique, a DNA strand having an unknown sequence of ~500 bases is isolated, cloned, and tagged at one end with phosphorus-32 (a beta emitter). Four identical aliquots of the DNA strand are then treated with selective chemical reactions that cleave the DNA strand at either the C, G, T, or A sites. The fragments derived from those reactions are placed in four lanes of the gel—one for fragments cut at the C base, one for those cut at the G base, and so on. The smallest of all the DNA fragments moves



The Sanger method for DNA sequencing is still in use today. (Source: Office of Technology Assessment, U.S. Congress.)

most rapidly under the influence of the electric field and, along with the others, is detected by autoradiography (images produced on X-ray film by beta radiation from the phosphorus-32). If the smallest fragment is in the C lane, then the first base in the DNA strand is a C. The lane location of the next smallest fragment (one base longer than the first) indicates the name of the second base in the strand, and the others are identified in the same manner. By reading the radiation band patterns on X-ray film of a gel, a technician can determine the base sequences (see figure on p. 33).

In the Sanger chain-terminating technique, the sequencing fragments are formed by incorporation of nucleotide analogs during DNA synthesis. These analogs prevent further elongation of the DNA chain during replication of the DNA segment of interest and form a random set of oligodeoxynucleotide fragments differing in length by one nucleotide. Like the Maxam-Gilbert technique, the Sanger manual sequencing technique requires several steps to complete the process.

Although sequencing methods based on radioactive labels are extremely powerful, they have several limitations. The radioactive isotopes used are unstable and expensive for large-scale applications, require handling by highly trained personnel, and may present a potential health hazard when disposed of as waste.

Fluorescence Detection

The classical sequencing methods are "off-line" because the fragment detection pattern is captured by a long-exposure film after the electrophoresis has run for several hours. Newer "on-line" methods detect the fragments as they move in the gel. Except for an EG&G system that detects the radioactivity of conventionally labeled DNA segments, most on-line systems utilize fluorescent tags.

Groups at the California Institute of Technology, E. I. du Pont de Nemours & Co., Hitachi of Japan, and the European Molecular Biology Laboratory (EMBL) have developed the partially automated "on-line" systems that use fluorescent dyes. In 1986, Applied Biosystems, Inc., of Foster City, California, developed the Caltech system into the first commercial automated DNA sequencer. This, and similar partially automated systems, can sequence 200 to 500 bases a day.

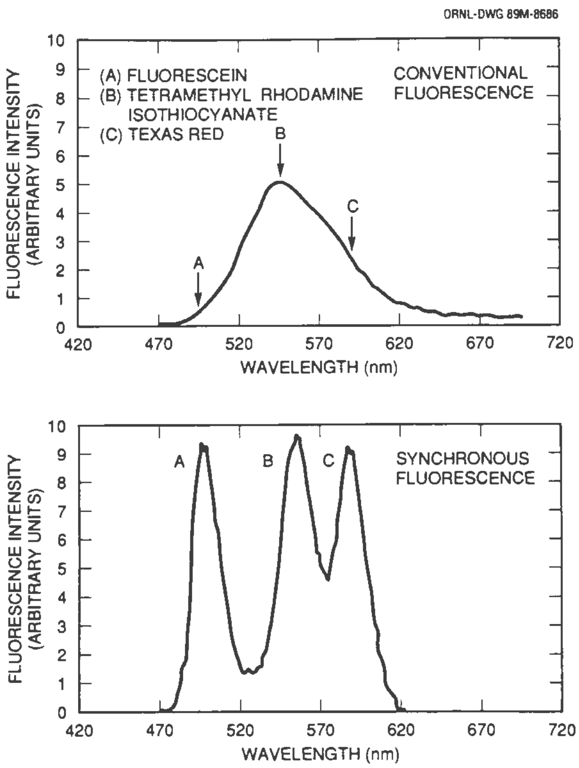
DNA fragments tagged with fluorescent dye molecules are detected by using an ultraviolet laser beam to activate the fluorescent dye labels and then measuring the light emission from each label. The fluorescent dye tags are covalently attached to the oligonucleotide primer used in enzymatic DNA sequence analysis. Four different types of fluorescent labels are used for each of the reactions—one specific to each of the four bases. The reaction mixtures are combined and moved by electrophoresis down a single gel lane. The fluorescence of the terminal base of each of the separated fragments is detected sequentially near the end of the lane, and a computer correlates the color of the emitted light with the appropriate base.

ORNL's Role

Developing new spectrochemical schemes. Since the recent research efforts in automation of DNA sequencing techniques and the use of fluorescence labels, limited progress has been made in developing advanced detection technologies for DNA sequence analysis. However, improved technologies are needed to minimize errors in fluorescence detection that result from problems in fluorescent labeling and gel resolution.

Improving the speed, accuracy, and efficiency of currently available fluorescence techniques for detecting bases is the goal of one ORNL research group. Tuan Vo-Dinh and his associates in the Health and Safety Research Division are examining the possibility of developing new spectrochemical schemes for DNA sequence detection and combining them in a new instrument. Their research is focusing on developing an integrated instrument based on hybrid multiplex systems, combining various spectrochemical detection schemes using fluorescence, phosphorescence, and Raman techniques.

One of the problems with the currently used fluorescence detection for sequencing is that the emission spectra of the different dyes overlap substantially. Because of this effect, emission



peaks corresponding to the presence of a single dye are detected in more than one channel, sometimes leading to sequence errors. Vo-Dinh and his associates in the Advanced Monitoring Development Group—Randy Johnson, Job Bello, and Henry Cheng—are developing new spectrochemical techniques that improve the accuracy of DNA base sequencing. In a recent experiment, they measured the conventional fixed-excitation and synchronous luminescence spectra of three different dyes—tetramethyl rhodomine isothiocyanate, fluorescein isothiocyanate (FITC), and Texas Red (TR)—used as fluorescent labels in DNA sequencing detection. Their recent success in obtaining the synchronous luminescence spectrum of the dye mixture (see figure above) shows that advanced detection techniques could improve the spectral resolution of the DNA base labels, increasing both the accuracy and speed of sequencing.

Detecting nonradioactive sulfur isotopes for sequencing. Another ORNL researcher is trying to improve the safety as well as the accuracy of DNA base detection. Bruce Jacobson of the Biology Division is working with Atom Sciences, Inc., of Oak Ridge on a project that should help meet two DOE goals: reduce the exposure of researchers to radiation and sequence the human genome. The problem is that many investigators using the radioisotopes phosphorus-32 and sulfur-35 may be exposed to increased radioactivity in their efforts to speed detection of DNA bases.

In the spring of 1988, Hal Schmitt, Atom Sciences president at the time, and Jacobson discussed the possibility of combining resonance ionization spectroscopy (RIS) with mass spectrometry for DNA sequencing. RIS, a laser-based technique invented at ORNL, is being used commercially by Atom Sciences, an ORNL spin-off company. The combined technologies can detect and quantify virtually all the elements and distinguish between each of their stable (nonradioactive) isotopes.

Once the question of using RIS and mass spectrometry for DNA sequencing was posed, the collaborators focused on the four stable isotopes of sulfur. If they could sensitively detect minute quantities of nonradioactive sulfur-32, sulfur-33, sulfur-34, and sulfur-36, these could be substituted for the sulfur-35 radioisotope normally used in sequencing. Calculations showed that RIS and mass spectrometry combined could detect stable sulfur isotopes at the same level (10 ^{15}mole) as with the radioisotope.

Why sulfur? First, the chemistry for labeling DNA with sulfur has already been developed. Isotopes of carbon, hydrogen, oxygen, and phosphorus were also considered for this reason but were discarded because they are normally present both in the DNA and the electrophoresis gel. Neither of these contains sulfur, so detection information is not obscured by background interferences.

In any DNA sequencing procedure, four types of fragments are produced, depending upon whether A, G, C, or T is the terminal base. Using the radioisotope technique, these four classes of fragments must be handled separately and compared after electrophoresis, because they are labeled by the same radioisotope.

During the conventional gel electrophoresis of the DNA fragments, the shorter fragments migrate faster and are more clearly separated than the

ORNL research shows that advanced detection techniques could improve the spectral resolution of fluorescent labels for DNA bases, increasing both the accuracy and speed of sequencing.

longer ones. The smaller fragments can be easily compared, but the large pieces are too close together to make meaningful lateral position comparisons across the four lanes, thus making accurate sequencing difficult. No more than 300 to 500 fragments per gel can be accurately analyzed (i.e., 300 to 500 bases is the maximum that can be sequenced in one experiment).

However, because four stable isotopes of sulfur are available, the terminal base of the four sets of DNA fragments can be assigned a specific isotope in the RIS/MS sequencing method, and then all fragments can be combined for electrophoresis in one lane, as in the fluorescent on-line method. Besides reducing gel usage, this approach should make it easier to obtain accurate information by avoiding lateral aberrations between lanes.

Can RIS and mass spectrometry resolve the larger, crowded fragments if they contain the four stable sulfurs? Probably so, Jacobson believes. In the first part of the analysis, the researchers plan to use either a sputtering beam or a laser to bombard the DNA sample. They calculate that by using RIS and mass spectrometry, stable sulfur isotopes, and one gel lane instead of four lanes, each experiment can be done faster and will provide more information than the conventional radioisotope sequencing method.

In October 1988, the researchers started experiments to determine the feasibility of their ideas. By the middle of 1989, they expect definitive results. Meanwhile, they are examining stable isotopes of many other elements that could be used to label DNA, because some of these can be detected 100 times more easily by RIS than the sulfur isotopes. To use these other isotopes, new chemical procedures for labeling the DNA will be necessary. Working out the new chemistry are Gilbert Brown, Bruce Moyer, and Richard Sachleben of ORNL's Chemistry Division. How to attach these isotopes to DNA is being examined by Bob Foote of the Biology Division. DNA fragment separation is being studied by Frank Larimer and Richard Woychik of the Biology Division. And RIS analysis is being adapted for DNA sequencing by Hal Schmitt, Heinrich Arlinghaus, and Norbert Thonnard at Atom Sciences, Inc. If the work succeeds, the investigators should increase the rate of DNA sequencing and eliminate the use of radioactive isotopes.

Two other ORNL researchers are working on developing new sequencing techniques, including one that detects modified DNA bases and one that eliminates use of gel electrophoresis.

Analyzing modified DNA bases. Today's DNA sequencing technologies are limited. For example, they are unable to distinguish between normal cytosine, a DNA base, and 5-methylcytosine, the modified, or methylated, version because of the methods used to prepare DNA fragments for sequencing.

The frequency and specific location (locus) of 5-methylcytosine within a DNA sequence have been shown to govern the expression of a wide variety of genes whose protein products are responsible for characteristics ranging from eye color to sex of the organism. Therefore, it is important to be able to distinguish between the methylated and nonmethylated cytosine bases to determine the part of the DNA sequence that might control gene expression.

In a practical sense, DNA sequences from different people can vary both from genetic differences and from environmental conditions. When cells are exposed to environmental chemicals known to damage DNA, the level of DNA cytosine methylation in daughter cells can be decreased significantly. Although disease cannot be predicted on the basis of methylation level, it is known that cellular characteristics change according to the location and amount of methylation on DNA. This kind of information will be valuable in the future use of DNA sequence changes as diagnostic tools. One goal of ORNL's work for the Human Genome Project is to develop chemical methods that will determine the location of this modified cytosine base in DNA. The project is being carried out by Mayo Uziel of the Health and Safety Research Division.

The approach Uziel and his co-workers will use to determine the positions of 5-methylcytosine residues in human DNA is based on a two-stage chemical process to remove the 3' terminal base from all the DNA molecules in the sequencing device. The two-step process provides a unique control mechanism for ensuring a predictable yield of terminal base per cycle, permitting computer-based reconstruction of the sequence even after several hundred cycles of sequential degradation. This technique will complement the existing DNA fragment analysis approaches to sequencing.

Toward gel-less sequencing. Accuracy in base determination is extremely important in DNA sequencing, because a single error in the sequencing of a gene containing thousands of bases could cause significant misunderstanding of the gene function. In addition, the correct sequence is important for subsequent studies.

The new on-line sequencing systems have an error rate of ~1%. This rate must be reduced by 100 to 1000 times (to ~0.001%), because the complete task of sequencing the human genome will require the identification of about 10^{10} bases. An error of 1% would lead to ~10^{8} sequence errors! However, because both strands of the DNA helix can be sequenced, the error rate would then be 1 in 10^{4}.

An advanced technique is believed necessary if DOE is to attain its goal of sequencing a million bases a day and sequencing all three billion base pairs of the human genome by the end of the century. Sequencing the genes of many other organisms such as bacteria, plants, and mice, could also add much to understanding human development and our relationships with other living organisms, further emphasizing the need for a better and faster sequencing method.

At ORNL, Jack Davidson of the Instrumentation and Controls Division is developing a new high-vacuum, laser-based technology to eliminate the time-consuming gel electrophoresis of conventional sequencing and provide much faster, more nearly accurate base resolution. If this concept of a gel-less sequencing system is successful, it will separate DNA fragments in about a millisecond rather than the hours now required by the most rapid partially automated fluorescence systems. The velocity of the DNA fragments will be measured by laser beams in a kind of "molecular steeple chase." Because the separation of DNA segments will be unimpeded by the interstices of the gel, it can proceed many times faster.

A number of problems must be resolved to make this approach practical. Fortunately, the broad interdisciplinary resources at ORNL are available to help work out what may be the next generation of DNA sequencing techniques.

Images of DNA. Finally, two ORNL researchers are using neutron and X-ray diffraction and supercomputer calculations to improve the visualization of segments of DNA and help resolve its three-dimensional structure (see sidebars on following pages). All these efforts should lead us closer to biology's "holy grail." ornl

DOE's Human Genome Information Management System Based at ORNL

An Information Management System for DOE's Human Genome Initiative has been established in the Information Research and Analysis Section of the Health and Safety Research Division (see Ensminger's article on p. 202). Betty Mansfield, who is setting up this system, is carrying out three tasks to inform employees of DOE and its contractors about the latest developments in human genome research: (1) producing a monthly *Human Genome Newsletter*, (2) setting up an electronic bulletin board, and (3) publishing quarterly technical reports on special topics.

3-D Structure of a Nucleosome

Chromosomes consist of DNA threads, which carry the genetic blueprint of an organism, and many associated structural and regulatory proteins. The basic repeating structural unit of the chromosome is the nucleosome, the object of our research effort. Using X-ray crystallography, Edward C. Uberbacher, a research assistant professor of the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, and I have produced a three-dimensional view of the nucleosome by determining its crystal structure.

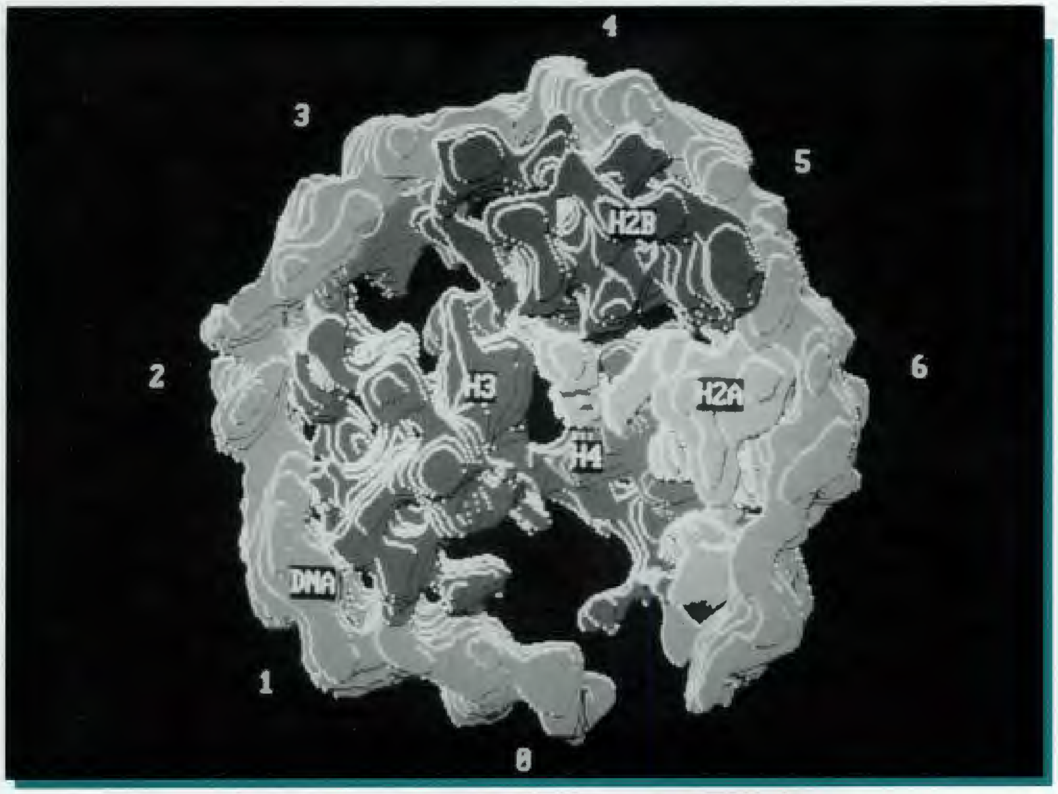
To isolate the nucleosomes for our investigations, we used a bacterial enzyme to digest chromatin—the material making up chromosomes—which we obtained from chicken red blood cells. The resulting core particles (i.e., the nucleosomes) consist of 146 base pairs of double-stranded DNA wrapped in 1.8 superhelical turns around pairs of the four histones that form the protein core (histone octamer) of the nucleosome. The nucleosomes were crystallized, and the crystals were subsequently examined in X-ray diffraction experiments, leading to a determination of the nucleosome structure.

In each experiment, we placed a nucleosome crystal (typically $5 \times 0.1 \times 0.05$ mm) in an X-ray beam. The X rays passing through the nucleosome crystal lattice produced a diffraction pattern yielding information about the structure. Using standard crystallographic computing techniques and an optimized starting model with data from more than 5000 diffraction measurements, we calculated the distribution of electron densities in the nucleosome to a resolution of 0.8 nm.

We found that the continuous double-stranded DNA segment winding around the nucleosome core is longer and bends more than any other DNA segments that have been observed at near-molecular resolution. The image of the structure was assembled by stacking many two-dimensional sections of the contoured electron density images together between glass plates to form the final three-dimensional electron density map. The photograph on the next page (also shown on the back cover) is a computer-generated, solid-surface representation of our nucleosome structure in which colors are assigned to differing electron density regions to clarify the locations and extent of the four histone proteins and the DNA.

In analyzing the electron density map, we determined that the nucleosome is 10.6 nm across the coiled DNA superhelix and that the histone core is 8.5 nm in its longest dimension. We also observed distortions in the DNA that facilitate its bending. In addition to the typical helical repeat, which is about every 10 base pairs in B-DNA, we found a 20-base-pair structural repeat in the DNA segment wrapping around the nucleosome core.

We have cloned several specific sequences of DNA strands that have special properties allowing them to be positioned in precise registration around the histone octamer. Our focus now is on investigating the interactions that occur between the histone octamer and these DNA fragments. By reconstituting nucleosomes from histone octamers and these DNA fragments, we may achieve more uniform nucleosomes for structural studies at an atomic-level resolution. We hope to be able to locate all the phosphate atoms in the DNA as well as the carbon, nitrogen, and oxygen atoms in each histone protein and in the DNA. Another goal is to identify the specific interactions between the DNA and the histone proteins.



This computer-generated, solid-surface representation of a portion of a nucleosome was derived from more than 5000 X-ray diffraction measurements. In this view, about one superhelical turn of the double-stranded DNA winds around four of the light histones that make up the protein core.

Because structure determines function, our studies of nucleosome structure will help determine the mechanism by which messages from the genetic code are regulated (i.e., turned on and off). The three-dimensionality of the structure is important, because genetic expression is affected not only by the sequence of bases in the genetic code of each cell but also by the intricate coiling and folding of the code-carrying DNA strands. Deciphering the structure of the nucleosome may be as significant to understanding how genes work as knowing the actual gene locations on chromosomes and the sequence of DNA bases within those genes.

This research has been conducted in part using X-ray and neutron facilities at the National Center for Small-Angle Scattering Research at ORNL. To obtain a more detailed understanding of the nucleosome's structure, we plan to acquire higher-resolution data from our reconstituted nucleosomes using a powerful tunable-wavelength X-ray beam at the National Synchrotron Light Source of Brookhaven National Laboratory. Our ultimate long-range goal is to understand how gene expression is controlled at the nucleosomal level.—*Gerard J. Bunick, Solid State Division.*

Computing Molecular Structures

Certain DNA sequences play a role in controlling gene expression even though they are located in regions of the chromosome that do not code for proteins. Their ability to turn genes on or off may be affected by chemical exposures. Understanding the crucial role of these DNA sequences in the human genome is one goal of the structural-resolution research I am doing in conjunction with Suse Broyde of New York University.

The sequence-dependent fine structure of DNA may contain the critically needed information—for example, a bend in the DNA helix axis may indicate a rearrangement of base pairs in the same sequence. This type of structural information can be obtained at atomic resolution by X-ray crystallography or high-resolution nuclear magnetic resonance spectroscopy. However, such data have been obtained with great difficulty and for no more than about a dozen structures.

Furthermore, certain DNA sequences in proto-oncogenes (pre-tumorigenic genes) are particularly susceptible to damage by pollutants such as the benzo[*a*]pyrenes in automobile exhaust, industrial emissions, and cigarette smoke; these chemical-induced mutations can initiate the cell transformation that begins the process of carcinogenesis. Again, the shape, or conformation, of the particularly susceptible DNA sequence and its adduct—a covalently bound carcinogen such as B[*a*]P—is likely to be critical in determining why these particular sequences are vulnerable to carcinogen attack and mutation. However, none of our available experimental methods have produced atomic-resolution views of such carcinogenic DNA adducts.

Broyde and I have developed computational procedures to calculate molecular views of DNA duplexes to the dodecamer level (12 base pairs of DNA), and our methods can easily be expanded to visualize twice the number of residues. We have performed minimized potential energy calculations using the Cray-2 at the Magnetic Fusion Energy Supercomputer Center of Lawrence Livermore National Laboratory. We use a build-up technique developed as a strategy to deal with the multiple minimum problem because the lowest energy form cannot always be obtained easily. Small DNA subunits, single-stranded and duplex dimers, and trimers are investigated first via global searches of the conformation space. Larger macromolecules (oligomers) are built up in our computer model by combining the smaller pieces or by incorporating the smaller subunits into standard A, B, or Z DNA conformations.

These methods have succeeded in a priori predictions for the standard A, B, and Z conformations of DNA residues and have produced molecular views of a number of carcinogenic DNA adducts (see figure). Our views are consistent with low-resolution spectroscopic solution data, although atomic-resolution data for these structures are not available. For example, our technique has visualized a DNA strand modified into a severe bend by a (+)*trans*-benzo[*a*]pyrene diolepoxide adduct at the DNA base guanine. —*Brian Hingerty, Health and Safety Research Division.*

Using a supercomputer, Brian Hingerty has helped develop this molecular model of a carcinogenic adduct attached to 8 base pairs of a DNA duplex.

Global Environmental Studies Center Established at ORNL

In May 1989, a Center for Global Environmental Studies was established in ORNL's Environmental Sciences Division. Robert I. Van Hook, associate division director of this division, was named coordinator. He reports to Chester R. Richmond, associate director for Biomedical and Environmental Sciences.

According to an announcement by Richmond, "The interdisciplinary program will address global environmental concerns including climate change, ozone depletion, deforestation, ocean pollution, desertification, biodiversity, and resource depletion.

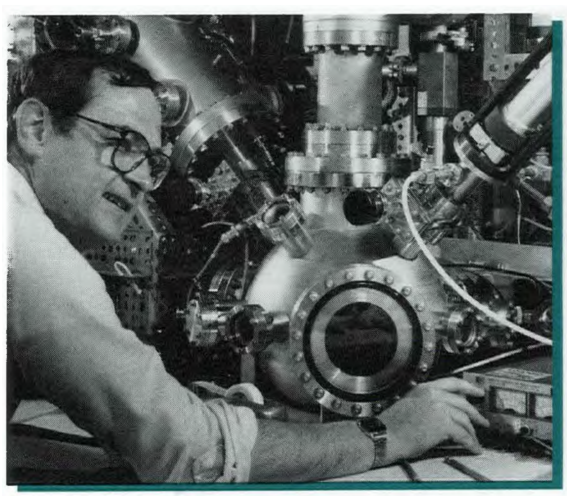
"The center's efforts will be directed toward environmental issues that are larger in spatial scale and operate over longer time periods than traditional environmental concerns. The program will build around a central theme of global systems analysis utilizing ORNL's strong analytical capabilities and extensive experience in applying systems approaches to landscape, regional, and continental-level environmental issues."

New Mass Spectrometry Lab Nears Opening

Although ORNL has some of the finest mass spectrometry capabilities in the United States, their usefulness in the past has been limited by inferior facilities and inaccessibility. This will no longer be the case when the new Mass Spectrometry Laboratory (MSL) opens its doors sometime in the summer of 1989. The new facility, located just south of Building 4500-S at ORNL, will not only be more accessible to Laboratory researchers, but will also be a "user-friendly" facility available to qualified students, professors, and industrial researchers on a collaborative basis.

Mass spectrometry is one of chemistry's most powerful analytical tools, using various technological approaches and instrumentation designed to separate chemical components of a sample on the basis of their mass and charge differences. Samples to be analyzed are first ionized, then accelerated through magnetic and electrostatic fields that deflect the charged ions, separating them according to mass and charge. Because some mass spectrometers can detect components at the parts-per-billion level, they are useful for characterizing materials, detecting airborne pollutants, and measuring isotopic abundances for reactor fuel certification or in spent nuclear fuel examinations. Extremely sensitive explosives detectors based on mass spectrometry are being developed and may someday prove useful for airport screening of luggage to prevent terrorist attacks.

The new, state-of-the-art MSL will eventually accommodate many types of mass spectrometry research, with instrumentation for organic, inorganic, isotopic, and surface analyses. It will support laser-based, secondary-ion, ion-trap, and Fourier-transform mass spectrometry operations. The initial 6200-ft^2 facility, which will be opened this summer, will house mainly organic mass spectrometry research. A 7300-ft^2 addition for inorganic mass spectrometry is scheduled for completion in 1990. Total cost for the MSL will exceed \$2 million, and DOE will invest \$1 million more in new instrumentation for the facility. ornl



Peter Todd adjusts an organic ion probe used for determining chemical distribution in tissue samples. This instrument will be in the new Mass Spectrometry Laboratory.

Radiation-Induced Cancers

By R. J. M. Fry

Radiation studies at ORNL have an illustrious past and continue to provide exciting cancer research information. Although the current program is not as large as in years past, its importance is perhaps greater. ORNL has the only U. S. external radiation carcinogenesis program that ranges from molecular studies to relatively large-scale, whole-animal experiments.

Radiation is a wonderful tool for investigating the general mechanisms of carcinogenesis. The various lesions induced in DNA by different types of radiation are known and can be quantified with fair accuracy. DNA repair mechanisms and their long-term effectiveness in speeding recovery from radiation damage are also well established.

Because the final pathways to cancer formation are common to all carcinogenic agents, much that is learned about radiation carcinogenesis can be applied to all cancer induction. A great advantage of using radiation is that the dose can be measured and manipulated better than that of any other cancer-causing agent. For example, most human exposures to radiation and chemicals occur at a low dose rate; in laboratory experiments, it is much easier to produce exact low-dose-rate exposures to radiation than to chemical carcinogens.

Our current studies are in two interrelated areas: (1) investigating the mechanisms of carcinogenesis and (2) providing experimental data to be used in making risk estimates and establishing radiation protection standards. The quality and breadth of the program are based on extensive collaborations both inside the Biology Division and with other institutions.

Extrapolating Cancer Risk

Evidence of common mechanisms for radiation-induced cancer among species would strengthen the conclusions John Storer and I have reached concerning the extrapolation of cancer risk across species (see article on page 44). Our studies indicate that mice are good models for estimating radiation-induced cancer risk in humans.

Data from animal experiments are also essential for estimating the cancer risks from exposure to neutrons and to heavy ions encountered in space, because no human data are available. Currently, the risk of developing cancer from neutron exposure is derived indirectly, by estimating effects of gamma rays and multiplying by a quality factor, *Q*, related to the characteristic energy deposition of the radiation. Currently, the selection of *Q* factors is, in part, a matter of personal judgment, based on experimental evidence. Because such decisions are judgment-based, differences of opinion inevitably arise.

To obtain more direct estimates, animal experiments are under way in our laboratory to determine the effect of small doses of fission-produced neutrons. If the Storer-Fry method of extrapolation across species is accepted, then direct, experiment-based estimates of risk could be extrapolated to humans.

How Tissues Suppress Tumors

Most, if not all, older people have altered cells with the potential to become cancerous but which never do so. Studies here and at other laboratories are investigating how various tissues suppress tumor development. Obviously, if we understood how the body controls the aberrant cells, we might be able to augment these processes to interfere with cancer development. Potentially cancerous cells can be identified by treatment with an agent that enhances the expression of malignancy or by providing a suitable environment. We also know that component cells of normal tissue behave as a controlled community, while dispersed cells show more individuality. Dispersed cells exposed to carcinogens, for example, are more likely to express their malignant potential.

Peggy Terzaghi-Howe and Bob Ullrich have developed in vivo–in vitro systems of epithelial cells that allow cell behavior to be studied both in animals and in laboratory media (see articles on p. 47 and p. 49). The in vivo aspects of these studies investigate the factors that promote and control cell growth. Cellular changes can be followed from the initial radiation-induced events through the progressive stages to malignancy. In addition, cells can be removed from an animal, grown in laboratory media, treated, and returned to the host animal. Whereas most cancer research is confined to the study of progressive changes in DNA, chromosomes, and cells, the technique of in vivo–in vitro studies has demonstrated that it is the later changes, some of which occur in the environment of potential cancer cells, that determine whether an overt cancer forms.

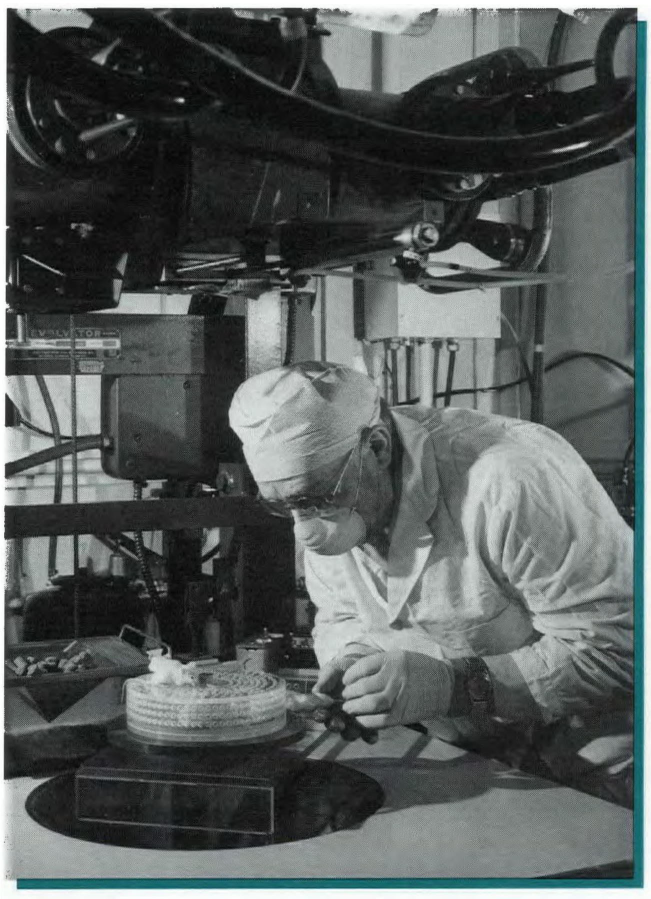
Leukemia–Chromosome Link

In their studies of mechanisms of radiation carcinogenesis, R. J. Preston and W. K. Yang are trying to identify the initial events, such as gene activation and chromosome aberrations, in four types of cancer. Although chromosome aberrations are associated with leukemias in both humans and mice, a causal relationship between the induction of specific chromosomal aberrations and the induction of a particular cancer has not been proved.

A myeloid leukemia comparable to a human leukemia type is found in certain strains of mice, providing a good model for studies of carcinogenesis mechanisms. Preston and his colleagues have found a specific chromosomal translocation (exchange of parts) in almost every case of myeloid leukemia in the few mice of the RFM strain that develop the disease spontaneously (even without exposure to radiation). The same chromosome aberration is found in the same type of leukemia occurring more frequently in irradiated mice (see Preston's article on p. 16). Interestingly, in humans having chronic myeloid leukemia, a specific oncogene (tumor-inducing gene) appears to be involved, and the same oncogene is also located on the chromosome associated with the leukemia in mice. Thus the mouse model provides the possibility of clinching the causal relationship biologists have sought. It may also make it possible to show whether the mechanisms for the induction of some cancers in humans are the same as in mice.

Our studies span the range of current research approaches to identifying the excess cancer risks to human populations exposed to various types of radiation, the natural controls of the cell's abnormal behavior, and the causes of cell malignancies. We also hope to convince our colleagues that the mouse model provides cancer risk data that can be reliably extrapolated to humans. ornl

R. J. Michael Fry is head of the Cancer Biology Section in the Biology Division. He came to ORNL in 1977, after receiving an M.D. degree from the University of Dublin in Ireland.



Mark Jernigan places mice in a holder before exposing them to X rays for cancer-risk studies

Extrapolating Cancer Risk

By John B. Storer and R. J. M. Fry

Experimental animals, particularly mice and rats, have been extensively used to show that a variety of chemical, physical, and biological materials can increase their incidence of cancer. Once the data are collected, however, no one knows or even has a strong clue about how the information can be translated into quantitative estimates of risk for a human population. About the best that can be done is to make "useful generalizations." For example, if a chemical causes cancer in mice, it is prudent to assume that it may cause cancer in humans.

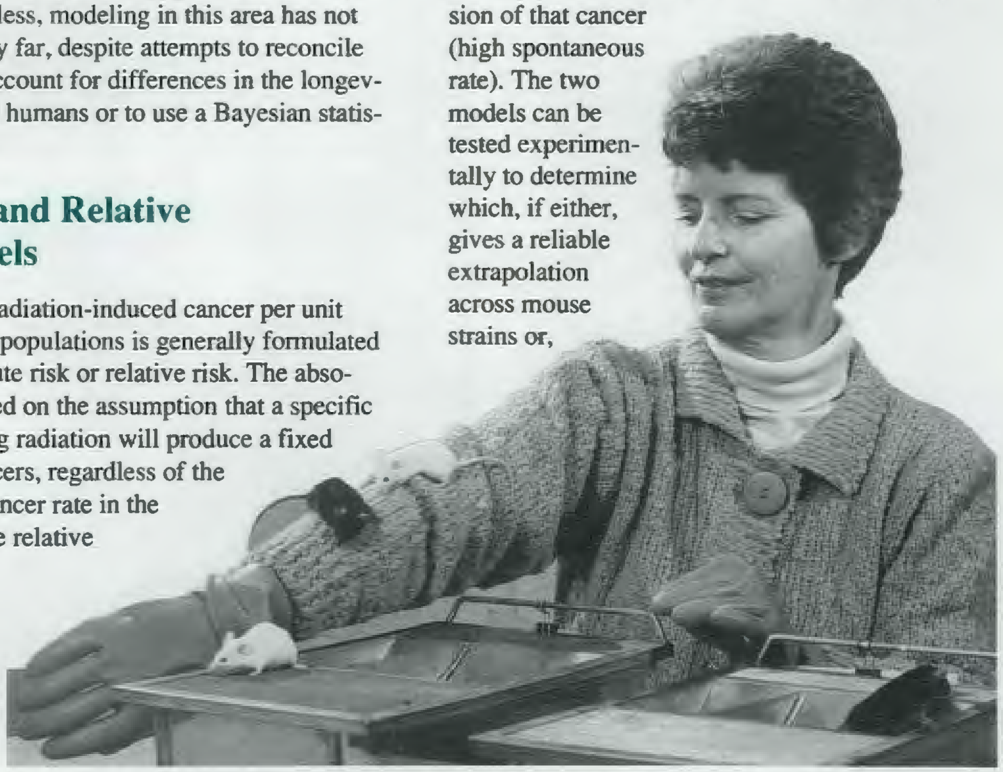
Ionizing radiation is the carcinogen that has been the most thoroughly studied. Because radiation doses can be accurately measured or estimated, it is possible to quantitatively estimate risk per unit dose for both animal and human populations. For chemical carcinogens, the dose that causes cancer in animals may be well established, but the cancer-producing dose for human populations is usually very poorly defined. For this and other reasons, radiation would seem the best choice for evaluating models for cancer risk extrapolation between or among species, including man. Nevertheless, modeling in this area has not progressed very far, despite attempts to reconcile age scales to account for differences in the longevity of mice and humans or to use a Bayesian statistical approach.

Absolute and Relative Risk Models

The risk of radiation-induced cancer per unit dose to human populations is generally formulated as either absolute risk or relative risk. The absolute risk is based on the assumption that a specific dose of ionizing radiation will produce a fixed number of cancers, regardless of the spontaneous cancer rate in the population. The relative risk assumes that the number of radiation-induced cases is related to the natural sensitivity or resistance of the population to cancer induction (as estimated by the spontaneous incidence) and that radiation, in essence, multiplies the spontaneous incidence. For the population under study, both methods of estimation necessarily yield the same answer in terms of excess, numbers of radiation-induced cancers.

When extrapolations are made from one population to others (e.g., from the Japanese A-bomb survivors to western populations), major differences in the estimates are apparent. Similarly, when forward projections are made of the expected incidence with increasing time after exposure, major discrepancies occur in the estimates. The uncertainty about which model to use contributes appreciably to uncertainties in the risk estimation for human populations. The data from human studies are not adequate to unequivocally reject either model.

We have long felt that the absolute risk model is inherently implausible because of its premise that the expected number of cancers is independent of the population's resistance to a particular cancer (low spontaneous rate) or sensitivity to the expression of that cancer (high spontaneous rate). The two models can be tested experimentally to determine which, if either, gives a reliable extrapolation across mouse strains or,



Lou Satterfield holds two inbred strains of mice that differ markedly in their genetic susceptibility to natural and radiation-induced tumors.

perhaps more important, if either can be used to extrapolate directly to humans. In the following, we describe the history and results of our research in testing the models.

A New Approach

In 1978, Michael Fry, a member of our research team, posed an interesting question: What would the estimates for human risk of radiation-induced cancer be if no data from human populations existed and if only experimental data had to be used, as is the case for estimating genetic risks? Fry then proposed that the relative risk estimates would be about the same for mouse and man. John Storer said that Fry was daft. Nevertheless, Fry persuaded Storer to develop risk estimates for tumors in those strains of mice for which adequate data had been accumulated.

We had sufficient data to derive estimates for three tumors, namely, acute leukemias and cancers of the lung and breast in irradiated female mice of the RFM and BALB/c strains. We then compared our risk estimates to those reported for the Japanese A-bomb survivors. The results, which amazed Storer and pleased Fry, indicated that the relative risks per unit dose in the mouse and human populations differed only slightly. We reported these results in oral presentations and included them in review articles. The results did not create much excitement, however, because we only had data for three tumors for female mice of two strains.

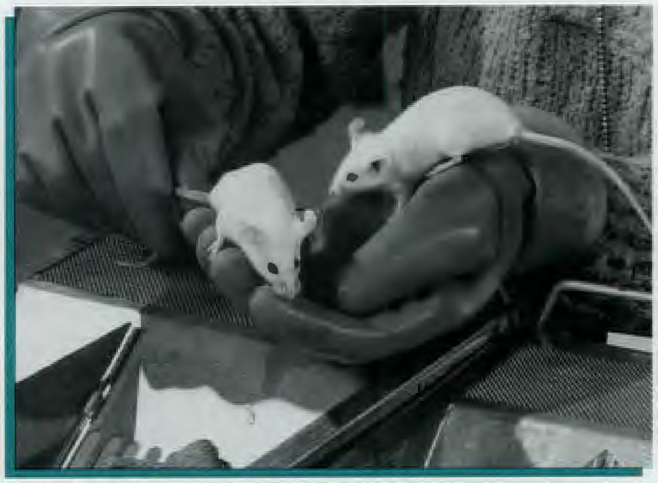
Testing the Hypothesis

To subject our hypothesis to a more rigorous test, we designed a study using male and female mice from two additional strains, which were known to develop a different spectrum of tumor types and which showed major variations in the spontaneous incidence of particular tumor types. This latter point is of crucial importance in assessing whether relative or absolute risk is the appropriate model for extrapolation. We also decided to evaluate primarily those tumors that caused death in the mice, making our data comparable with the Japanese data. By reviewing all the individual autopsy records for the two strains studied earlier and classifying all the tumors as incidental or fatal, we would thus have samples of mice for females of four strains and males of two strains. Our colleague, Toby J. Mitchell of the Engineering Physics and Mathematics Division, developed a mathematical model for rigorous testing of the extrapolation of absolute or relative risk of the various tumors among the mouse strains.

Normally, this type of study takes about ten years from initial concept to final publication; we completed our study in only eight years (1980 to 1988). The National Cancer Institute provided support, but it took time to get into the funding cycle. We then built up stocks of mice that were irradiated at the appropriate ages (by Mark Jernigan of the Biology Division) and then were returned to the animal facility. There Biology Division members Lou Satterfield and Norman Bowles checked the cages daily for dead or moribund mice for four years (some mice of these strains normally live that long) and autopsied all the animals to determine tumor types and causes of death. Because of their extreme dedication and diligence, we obtained autopsy information on over 99% of the mice. Further, we also obtained histological verification of the tumor types for more than 98% of the animals. The final year was spent doing data analysis and preparing our work for publication. Our report, "Extrapolation of the Relative Risk of Radiogenic Neoplasms Across Mouse Strains and to Man" (by J. B. Storer, T. J. Mitchell, and R. J. M. Fry) was published in the May 1988 issue of *Radiation Research*.

"Whatever causes an animal (including man) to be susceptible or resistant to the spontaneous development of a particular cancer also determines its sensitivity to cancer induction by exposure to radiation."

What were the results of the experiment, which included data for 9763 mice? Eleven types of fatal tumors occurred in sufficiently high incidence in two or more strains to allow testing of the extrapolation models. Two tumor types did not show an increase with radiation exposure (neither are they increased in human populations). For seven of the remaining nine tumor types, our data showed that the absolute risk model could be unequivocally rejected as the appropriate method for extrapolation. For the two tumor types for which absolute risk was not rejected, the spontaneous incidence in the different strains was similar.



Relative Risk Model and Extrapolation

The relative risk model, on the other hand, provided a good fit to the data in seven of nine cases. In the two cases for which it was rejected, the absolute risk model was also rejected. We believe we understand the reasons why neither extrapolation model fit these two types of tumors, but our speculations lie beyond the scope of this report. We concluded that the relative risk model will extrapolate the risk of radiogenic cancers between and among mouse strains, but the absolute risk model will not. Thus, the susceptibility to radiation-induced cancer is a function of the natural (spontaneous) incidence of that cancer in mice.

Relative risk of death from various neoplasms for Japanese atomic bomb survivors and for mice.

(For 1.0 Gy absorbed dose)

Neoplasm	Mice	Japanese survivors
Carcinoma of the lung	1.77	1.66
Carcinoma of the female breast	2.00	1.86
Leukemia	5.38	6.27
Malignant lymphoma	1.00	1.00
Hepatocellular (liver) carcinoma	1.37	1.74

For five mouse cancers, corresponding risk data exist for radiogenic cancers in human populations. We have used published reports on the Japanese survivors for the comparison shown in table below. We first, however, converted the reported doses for the Japanese (T65D—kerma free in air) to absorbed dose, to make the dosimetries comparable.

The risk estimates for these cancers in mice and humans are not significantly different (see table). The 95% confidence interval (not shown) for each species overlaps the risk estimate for the other. We conclude, therefore, that for tumors of homologous tissues, the relative risk values, so far developed from data for mice, are accurately predictive of the human risk. This direct extrapolation requires no adjustment for differences in longevity or differences in the spontaneous tumor incidence. It also seems to us that a fundamental message about tumor biology is contained in this result. Whatever causes an animal (including man) to be susceptible or resistant to the spontaneous development of a particular cancer also determines its sensitivity to cancer induction by exposure to radiation. ornl

John B. Storer, M.D., is a former director of ORNL's Biology Division, a Corporate Fellow (named by Union Carbide Corporation), and a recipient of the E. O. Lawrence Memorial Award. He received his doctorate in medicine from the University of Chicago and has served on many prestigious scientific committees.

R. J. Michael Fry, M.D., is head of the Cancer Section of the Biology Division. He received his doctorate in medicine from the University of Dublin, Ireland. Before coming to ORNL, he worked at Argonne National Laboratory and the University of Chicago.

In Vivo–In Vitro Models for Cancer Studies

New model systems aid understanding of cancer mechanisms.

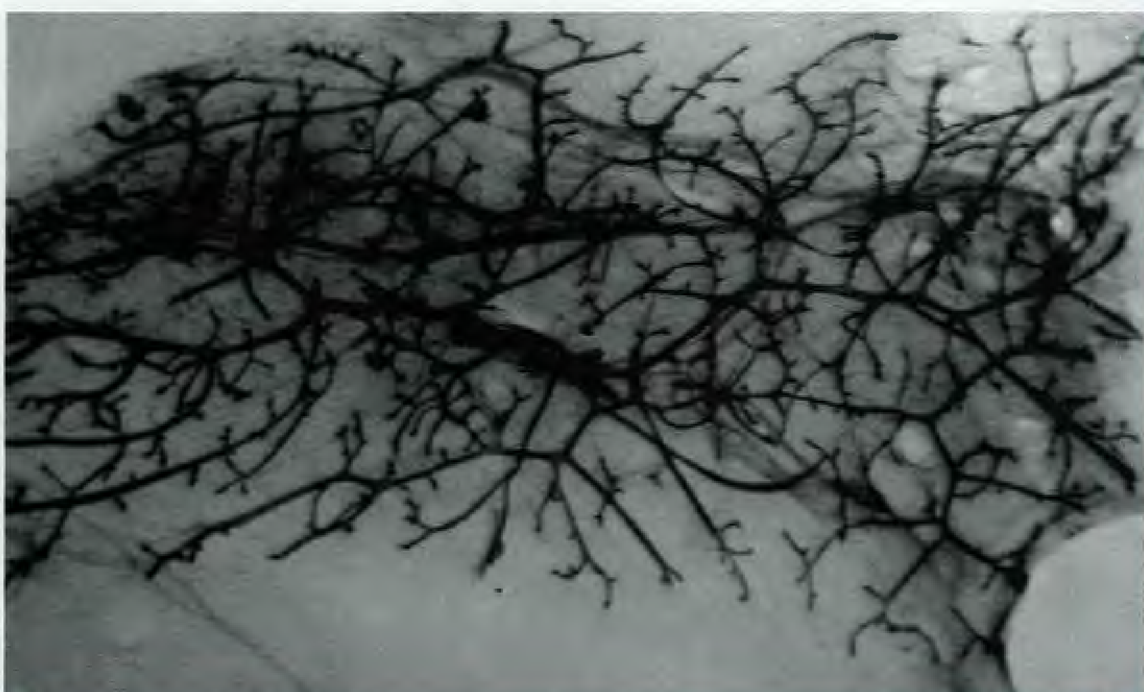
By Robert L. Ullrich

For several decades, the Biology Division has been a leading source of research data on the cancer-causing effects of ionizing radiation. Experiments here have provided much of the information available on the relationship between radiation dose and cancer risk and on the influence of dose rate on this risk. By determining the frequencies of specific cancers in mice after their exposure to radiation delivered at various levels and dose rates, we have gained some understanding of these quantitative relationships that has also been useful in evaluating human cancer risks. We still need to improve our understanding of the mechanisms involved.

Scientists want to know which specific changes in mammalian cells produced by radiation exposure are responsible for the development of cancer. Such information cannot readily be obtained from studies of cancer development in intact animals. Because many weeks or months elapse between the radiation exposure and cancer development, any detection of the early cellular alterations involved is very difficult.

Host Factors

In vivo (live animal) studies clearly show that cancer development is very dependent on the cell's environment. For example, the risk for cancer development may be markedly different between male and female mice and among mice having differing genetic backgrounds. This indicates that, to understand cancer-causing mechanisms, it is important not only to determine the cellular changes involved but also to understand how secondary "host factors" influence the early transformation of normal cells into tumor cells. Although the role of host factors can be inferred from in vivo experiments, the mechanisms by which these factors enhance tumor development cannot be explored using in vivo



This apparently normal mouse mammary gland is, in fact, a "reconstituted gland" grown from cells injected in fat tissue. Using this same technique, researchers can detect the neoplastic changes that occur in tissue grown from injections of irradiated cells.

systems because the various components involved cannot be isolated and individually controlled.

One approach to examining cellular changes has been the use of cell lines or primary cell isolates grown in culture. Such in vitro analysis allows the examination and quantification of many radiation-induced cellular changes. The difficulty with in vitro systems is that they do not allow us to study the influences on tumor production of secondary host factors and the cellular environment within the intact animal.

We have developed two in vivo–in vitro epithelial model systems—the rat trachea model and the mouse mammary model. In these model systems, tracheal or mammary cells are irradiated in the intact animal (in vivo). These cells can then be isolated from the animal and cultured in vitro at various times after irradiation. Cellular changes can be identified from the in vitro growth characteristics of the isolated cells.

For example, we can test the ability of radiation to induce cells to grow in culture under conditions in which nonirradiated normal cells will not grow. We can quantify cellular changes and follow the dynamics of cancer development in vivo by isolating cells and examining their in vitro growth characteristics, along with the accompanying cellular and molecular alterations. These cells can, in turn, be reintroduced into their normal in vivo environment to determine their in vivo growth and tumor-producing potentials.

Model Uses

These in vivo–in vitro model systems allow us to

- quantify and study early radiation-induced cellular and molecular changes involved in the process of tumor formation,
- monitor the process dynamics of cancer development as it occurs in vivo because, during the time cells are left in the intact animal, they maintain their normal relationships and interactions with their environment, and
- use the advantages of both in vivo and in vitro systems and overcome the disadvantages of each.

Using this system, we have observed that radiation induces, almost immediately, the transformation of a high percentage of normal cells into potential cancer cells. Shortly after irradiation, these cells are not cancerous but have a high probability of eventually developing into cancer cells. This cannot be detected in the intact animal, however, because of cell-cell interactions that appear to suppress the expression of their tumor-producing potential. The alterations that make the cells cancerous can be detected only if the cells are isolated and maintained in vitro or injected into host mice. The altered cells then appear to undergo a series of progressive changes that ultimately result in cancer cells.

Our studies have clearly shown that the progression and expression of these cell alterations are controlled to a large extent by host factors that are poorly understood. The study of these factors is another facet of ORNL research, which is discussed in the article that follows. ornl

Robert L. Ullrich, formerly a research staff member and leader of the Radiation Carcinogenesis Group in ORNL's Biology Division, is a professor in the Department of Radiation Therapy, Division of Research, University of Texas Medical Branch, Galveston, Texas. In 1987 he received the Radiation Research Society Award.

Probing Mechanisms for Cancer Suppression

An ORNL technique improves understanding of factors that block tumor growth.

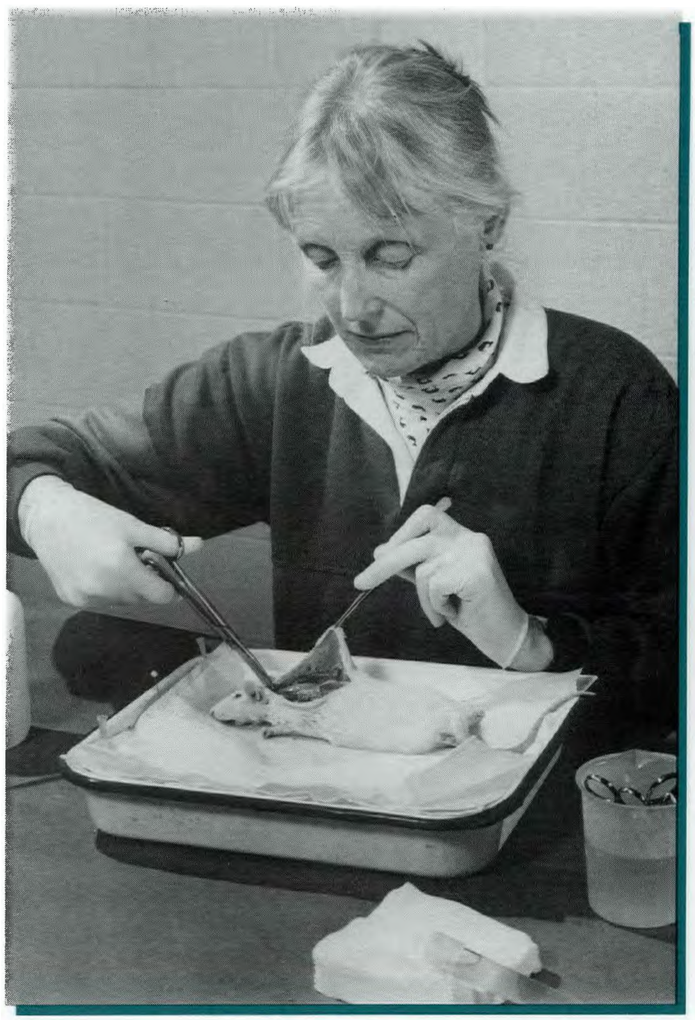
By Margaret Terzaghi-Howe

Understanding what makes a cell cancerous can come only from understanding what makes most cells normal. Typical normal tissues and organs exhibit continuous orderly patterns of cell growth and differentiation. The differentiated cells do not proliferate but serve a particular function, specific for each tissue or organ. This carefully regulated process is presumably the result of a precise balance between positive and negative growth regulatory factors within each tissue. When the delicate growth regulation balance is disturbed, the result can be an uncontrolled growth of cells—a cancer.

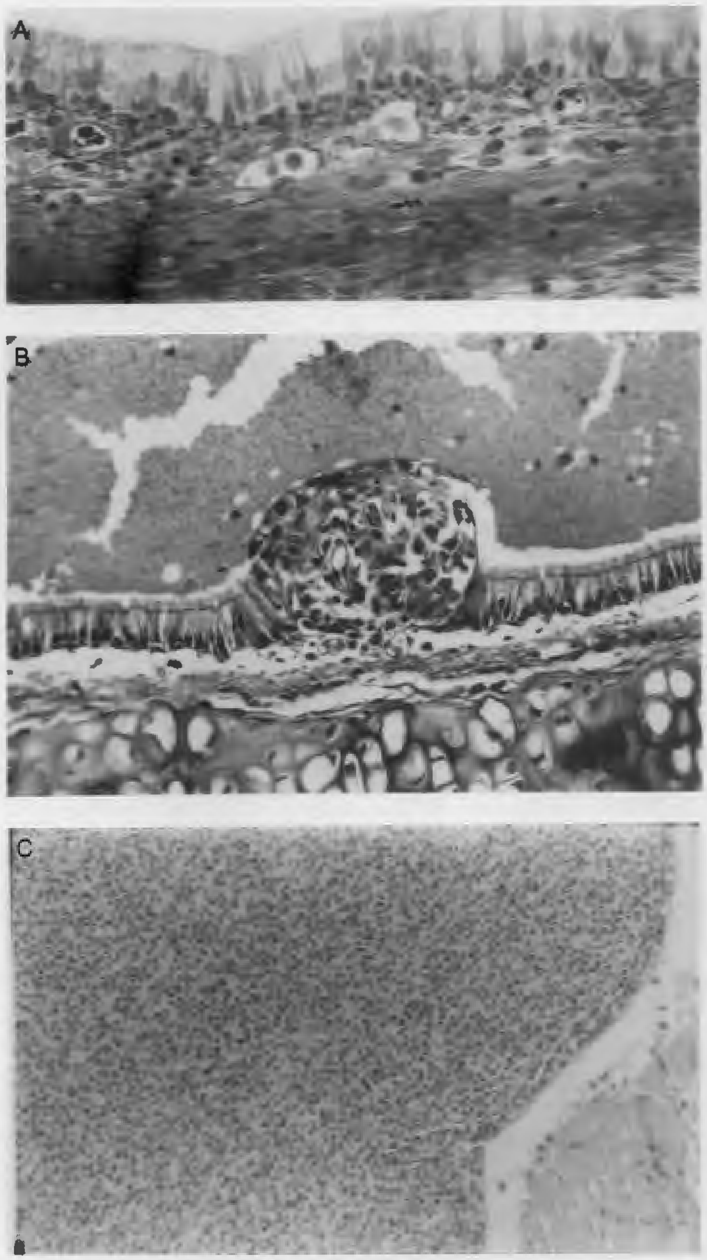
Following the exposure of a population to a carcinogen (radiation or chemicals), a relatively small fraction of exposed individuals will ultimately develop tumors years later. In contrast, when isolated populations of cells growing in culture are exposed to comparable doses of carcinogens, many of these cells have the potential to produce tumors. These observations taken together suggest that some mechanism or mechanisms in the intact tissue may be operating to eliminate or control potentially tumorigenic cell populations.

Rat Trachea Experiments

In our laboratory, we seek to define the mechanisms involved in the suppression of tumor development in intact tissues. For these studies, we use rat tracheas exposed to chemical carcinogens or radiation. Our initial experiments were designed to test the hypothesis that some attribute of the intact tissue or host environment could, in fact, inhibit tumor development in our model system. Combined cell culture (in vitro) and intact animal (in vivo) experiments were carried out. Tracheas were exposed in vivo to a dose of carcinogen found to yield a 10% tumor incidence during the normal animal life span. At various times after exposure, tracheas were removed from the host animals, cells were harvested enzymatically, and the levels of potentially neoplastic (tumor) cells per trachea were determined (see Ullrich's article on p. 47). Potentially tumorigenic cells were detected by seeding enzymatically dispersed cells into culture



Peggy Terzaghi-Howe surgically inspects trachea that were subdermally implanted in this mouse after inoculation with test cells. The technique helps clarify the interactions of various cell populations in an environment similar to that of the intact trachea.



Histologic sections of repopulated tracheas:

(A) Trachea inoculated with 5×10^4 normal tracheal cells. The ends were tied off, and the trachea was transplanted subdermally for 4 weeks. This section exhibits normal tracheal morphology.

(B) Same as (A) except in addition to 5×10^4 normal tracheal cells, 10^5 tumor cells were inoculated prior to transplantation. Isolated islands of tumor cells were detected, but no tumors developed.

(C) Tumor cells (10^5) were inoculated into a trachea in which one-third of the normal epithelium was removed. Under these conditions tumor cells are not inhibited by the remaining normal cells, and an invasive tumor has totally disrupted the normal tracheal architecture.

dishes. Isolated single cells were allowed to proliferate for several weeks. The progeny of the single cell formed isolated colonies that could be subcultured separately and ultimately tested for tumorigenic potential. Virtually all exposed tracheas contained >100 potentially tumorigenic cells per 10^6 cells.

Based on the tumor data for the same exposure, roughly 1 tumorigenic cell per 10^7 cells would be expected. Under the same culture conditions, no tumorigenic cells were detected in control non-exposed tracheas. These data clearly support the hypothesis that the behavior of potentially tumorigenic cells is, in fact, modulated by some mechanism(s) in the intact rat trachea.

Inhibitory Mechanisms?

Several mechanisms could be holding potentially neoplastic cells in check in the intact tissue. The following hypothetical mechanisms could be involved: (1) factors that govern normal cell growth and differentiation may also influence potentially tumorigenic cells, and (2) cells within a tissue communicate with each other and thus may control growth in adjacent cells.

Normal cells may produce some stable inhibitory substance that can diffuse to and affect the growth of other cells within the same organ. This mechanism would not require direct contact between normal and altered cells. If a short-lived inhibitor were produced, thus limiting the area of diffusion, proximity between the inhibiting and the inhibited populations would be a requirement.

Another possibility is that inhibitory signals are transmitted directly between contacting cells. Cells within a tissue have some similarities to a single cell, because they are joined by hollow-gap junctions through which small (<1000 mol. wt.) growth-regulatory molecules can be transmitted. Inhibition of potentially neoplastic cells through this mechanism would clearly require direct cell contact. We are currently carrying out experiments designed to differentiate between these alternative hypothetical mechanisms.

Tracheal Repopulation

A valuable tool in these investigations is "tracheal repopulation," a technique developed in our laboratory. The intact tracheal epithelium is partially or totally destroyed, and mixtures of normal carcinogen-altered cells are then inoculated into the tracheal lumen. The ends of the trachea are tied off, and the trachea is transplanted under the skin on the back of another animal. In this new location, blood vessels grow into the transplant and the inoculated cell mixture generates a new tracheal lining. Using this method, we can artificially reconstitute the tracheal lining with a known mixture of altered and normal cells.

We can further influence the spatial relationship of these cells by only partially denuding the trachea of its normal epithelium and then inoculating neoplastic cells. The neoplastic cells grow only on that portion of the tracheal transplant devoid of normal epithelium. However, when carcinogen-altered cells mixed with an appropriate number of normal tracheal cells are inoculated, the reconstituted tracheal epithelium will consist of carcinogen-altered cells interspersed with normal cells. Under these conditions, tumorigenic cells do not grow or develop into tumors but remain dormant. On the other hand, when tracheal repopulation is carried out such that tumor cells occupy a contiguous segment adjacent to a contiguous segment of normal cells in the reconstituted tracheal lining, no influence or inhibiting effect of normal cells on tumor development is observed. These data suggest that either direct cell-cell contact is required for tumor cell inhibition or any diffusible inhibitor produced by normal cells must have a very short-range effect.

We have started cell-culture studies to evaluate the relative importance of direct cell contact and diffusible factors in the normal-cell-mediated inhibition of tumor cells. If a diffusible inhibitor is involved, it could possibly be demonstrated in a culture medium harvested from normal cells. When a normal-cell-conditioned medium was placed on cultured preneoplastic cells (those that will eventually progress to a tumorigenic state) inhibition of growth was, in fact, observed. However, when the conditioned medium was placed on neoplastic (tumorigenic) cell cultures, no inhibition was observed. When the neoplastic cells were co-cultured in the same dish with normal tracheal cells, however, inhibition was observed.

Defining the Balance

Now that we have what appears to be a reasonable cell-culture model for events occurring in the intact tissue, we are attempting to define the roles of identifiable diffusible inhibitory factors and cell-cell communication in the observed moderation of tumor development in the intact tissue.

Clearly, a delicate balance exists between controlled and uncontrolled growth in intact tissue. Many potentially tumorigenic cells are held in check, presumably by some of the same mechanisms that normally govern orderly tissue-specific patterns of growth and differentiation. With a better understanding of the factors that effectively control most carcinogen-altered cells within a living tissue, the development of cancer therapies that are more specific and less toxic than those currently used may be possible. ornl

Margaret Terzaghi-Howe has been a research staff member in ORNL's Biology Division since 1975. She has a DSc degree in radiation biology from Harvard University.

Protein Engineering

By Fred C. Hartman

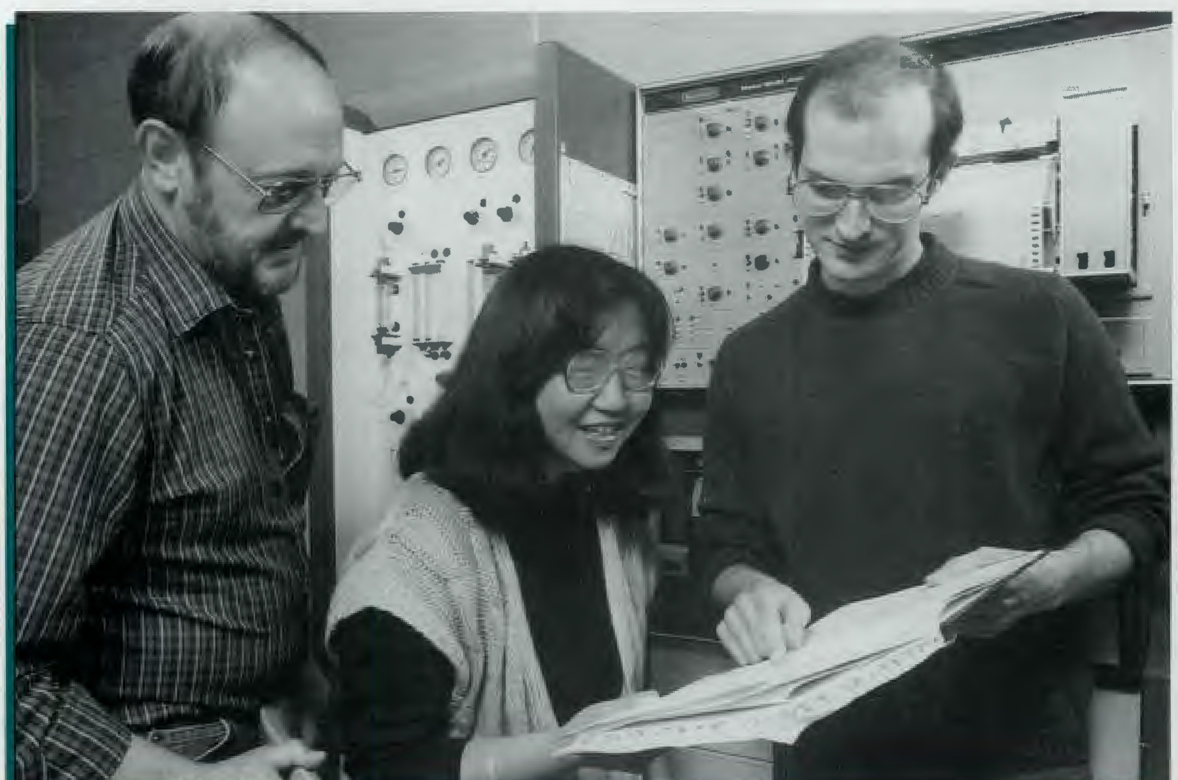
The diversity of protein function has intrigued and challenged biochemists for decades. Unlike the other two major classes of biological macromolecules having vital, but limited, functions (i.e., carbohydrates for energy storage and nucleic acids for propagation of species and as blueprints for cellular constituents), proteins serve crucial roles in all life processes. This multifunctionality of proteins is particularly profound, because all proteins consist of the same 20 basic building blocks (amino acids) coupled in a varying linear array. Despite this common structural mode, the properties of individual proteins differ dramatically.

Fortunately, some proteins are water insoluble (e.g., hair, skin, muscle, collagen) and act as the "glue" that holds an organism together and prevents it from dissolving in the rain. The functionally more dynamic proteins are generally water soluble. These include enzymes, the catalysts for the thousands of chemical reactions ongoing in all living cells; hormones, the regulators of metabolism; antibodies, the defenders against invasive agents; transport proteins, the carriers of nutrients from location to location within the organism; and nucleic-acid–binding proteins, the regulators of gene expression.

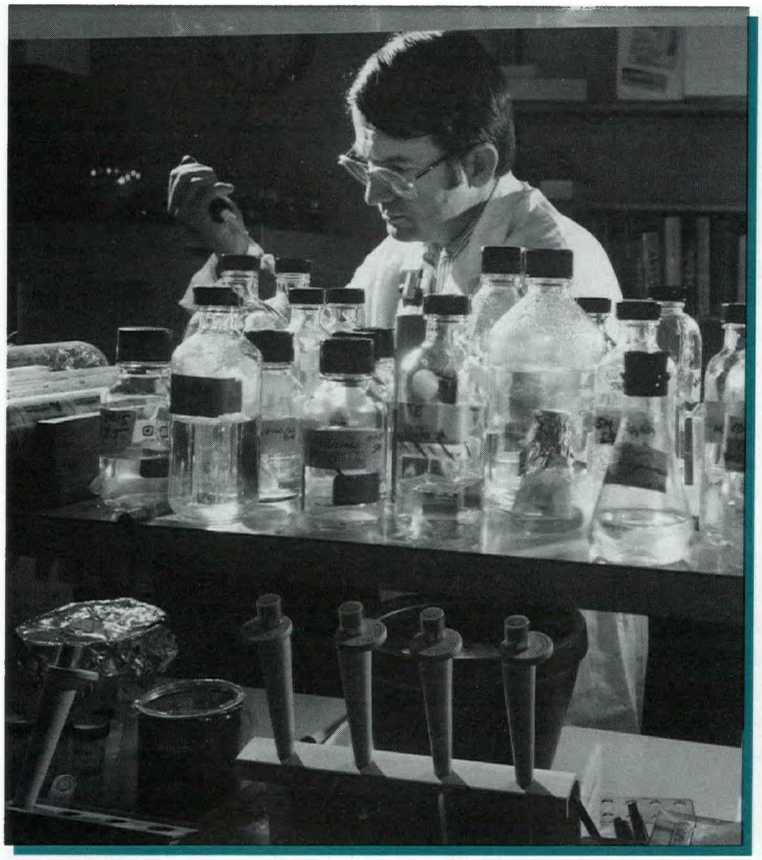
Proteins and Genes

A central goal of modern biological research is to explain protein function in terms of structure. Consider a protein molecule as a miniature machine having hundreds of operating parts; if we equate each part with an amino acid, remove each amino acid, one at a time, or replace each one with a different amino acid, it should be possible to critically evaluate the function of part of the protein machine. In fact, recombinant DNA technology provides these very capabilities.

The precise alignment of amino acids within a protein is dictated by the linear sequence of the



Claude Stringer, Eva Lee, and Harry Smith take a look at data collected in their protein characterization work.



Although advanced technologies are used whenever possible, protein engineering is largely carried out by hand, as Frank Larimer demonstrates.

four basic building blocks (bases) within deoxyribonucleic acid (DNA). A segment of DNA that encodes the sequencing information for one protein is called a gene. Thousands of genes linked in tandem form the complete DNA molecule—a chromosome. The human cell contains 23 pairs of chromosomes, and thousands of biologists are busy determining the exact arrangement of genes within each chromosome, in the well-publicized, multibillion-dollar, DOE-stimulated, national "genome sequencing and mapping initiative."

Recombinant DNA technology enables researchers to move genes from one organism to another and to specifically alter the base sequence of genes, thereby providing an avenue for instructing cells to make proteins never before produced in nature. The systematic alteration of amino acid sequences in proteins by gene manipulation represents a new frontier in biological chemistry called protein engineering. In the scant eight years since its inception, protein engineering has emerged as the most powerful tool for addressing mechanistic questions about protein function. In the longer term, protein engineering offers an exciting approach to optimizing properties of proteins for particular applications. If enzymes can be made more resistant to high temperatures, oxidation, and degradation, their uses in medicine, agriculture, food processing, waste treatment, detergents, and bioprocessing will be greatly expanded.

Since its initiation in 1984, and with partial support provided by the ORNL Director's R&D Fund, the Biology Division's protein engineering program has become a major focal point for DOE-sponsored biochemistry and molecular biology research. At ORNL, we are intensively investigating (1) the epidermal growth factor, a hormone that regulates cell proliferation and differentiation (see Niyogi's article, p. 54); (2) a DNA repair protein, which mitigates mutagenic and carcinogenic effects of some toxic chemicals (see Mitra's article, p. 57); and (3) the carbon dioxide fixation enzyme, a major determinant of biomass yield (see my article on p. 60). If we are successful in our protein engineering projects, the benefits could be enormous. ornl

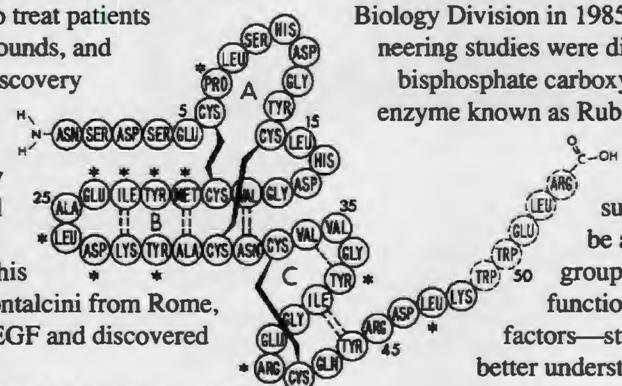
Fred C. Hartman, a senior scientist and director of the Biology Division, joined ORNL in 1966. He holds a Ph.D. degree in biochemistry from the University of Tennessee, Memphis, and has received honors that include the Pfizer American Chemical Society Award in Enzyme Chemistry, a Martin Marietta Energy Systems Technical Achievement Award, and two Energy Systems publications awards. Energy Systems recently named him a Corporate Fellow.

Altering Human Epidermal Growth Factor

By Salil K. Niyogi and Audrey Stevens

Regulating the growth of cells and tissues inside the body is an extremely important, finely tuned process requiring the action of growth factors. These relatively small proteins control cell growth by communicating information from the outside to the inside of the cell. Some types of cancer are known to be caused by a breakdown in communication, resulting in uncontrolled cell growth. Studies of growth factor proteins are leading to an understanding of the mechanisms by which molecules regulate growth and may ultimately guide the development of ways to restore control of cell growth in diseases such as cancer.

Among the most highly studied growth factors is epidermal growth factor (EGF), a small protein containing 53 residues of the 20 possible amino acids that are the building blocks of proteins (see figure). Because it promotes rapid cell growth, EGF is used clinically to treat patients suffering from burns, wounds, and gastric ulcers. For his discovery and subsequent study of EGF, Stanley Cohen of Vanderbilt University received the 1986 Nobel Prize in Medicine and Physiology. He shared this prize with Rita Levi-Montalcini from Rome, Italy, who also studied EGF and discovered nerve growth factor.



Schematic representation of the secondary structural features of human EGF [adapted from Cooke, et al., *Nature*, **327**, 334–37 (1987)]. Asterisks indicate targeted amino acid residues; solid lines indicate disulfide bridges; and dashed lines indicate hydrogen bonds.

A number of recently identified cellular genes called proto-oncogenes are involved in the control of normal cellular proliferation and differentiation. If we can understand what activates these structures as oncogenes (tumor-causing genes), we will know more about both the initiation and maintenance of cancer. Evidence linking growth factors with cancer has come from recent studies that link oncogene-encoded products with growth factors and their receptors.

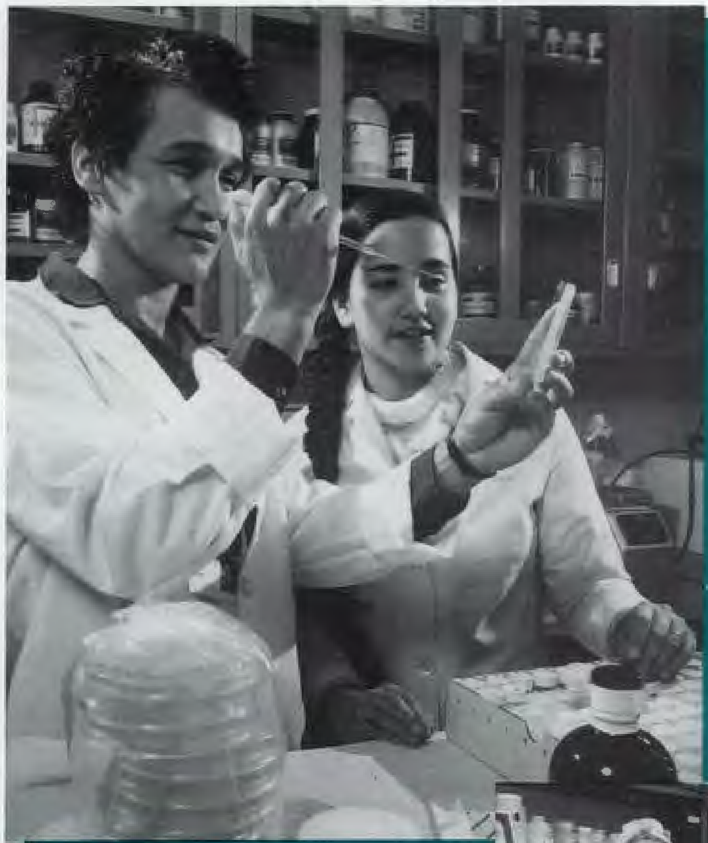
EGF (6000 mol. wt.) regulates cell growth by first binding to a specific cell-surface receptor—the EGF receptor. This binding stimulates the receptor's tyrosine kinase activity (phosphorylation of the amino acid tyrosine in proteins), which unleashes a cascade of biochemical events leading ultimately to controlled cell growth and proliferation. The oncogene *v-erb*B, however, codes for a product homologous to a portion of the EGF receptor in which both the EGF-binding domain and another small domain have been deleted. Such structural changes may contribute to the transforming potential of this oncogene and, thus, to uncontrolled growth.

A great volume of research on EGF has been performed worldwide over many years. Yet very little was known about the amino acid residues that are crucial for EGF's all-important binding to its receptor and stimulation of the receptor's tyrosine kinase activity that controls cell growth. When the Molecular Mutagenesis and Protein Engineering Program was established in ORNL's Biology Division in 1985, our initial protein engineering studies were directed at ribulose-bisphosphate carboxylase, the photosynthetic enzyme known as Rubisco (see Hartman's article on p. 60). In 1986, Audrey Stevens suggested that EGF would be an ideal target for the group's studies of structure-function relationships of growth factors—studies that might lead to a better understanding of cancer.

Cloning and Altering the EGF Gene

For our studies of EGF, we have used protein engineering—the replacement of a specific targeted amino acid residue in a protein with another amino acid. By replacing an amino acid believed to be essential to a protein's function and examining the biological consequences of the change, we can determine whether (and sometimes how) amino acids in specific positions are crucial to the normal functioning of the molecule. To achieve

ORNL biologists have cloned genes to produce growth-regulating proteins that may prove useful in treating cancer.

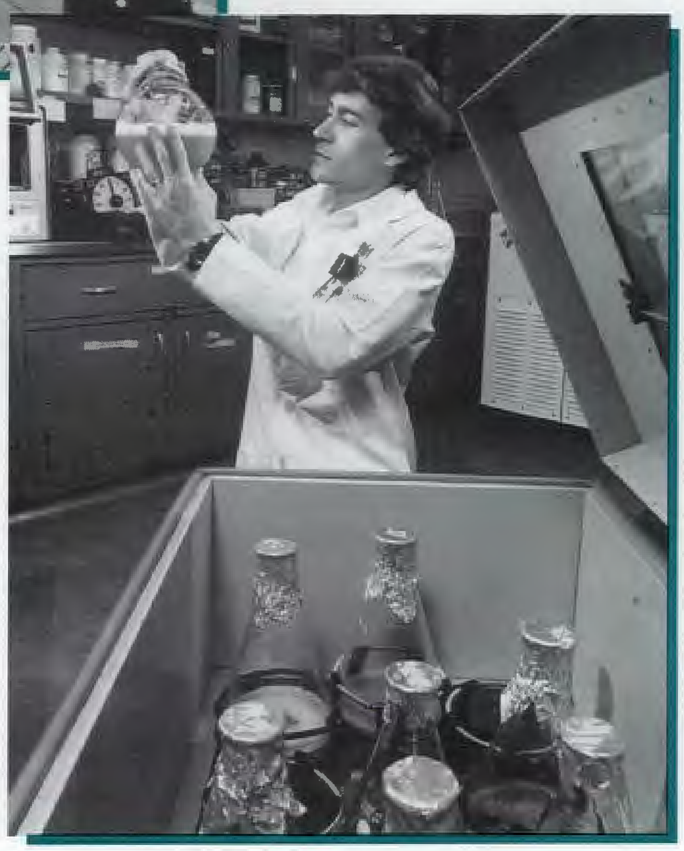


Risë Matsunami, graduate student, observes Margaret Yette, technician, demonstrating how to remove recombinant epidermal growth factor clone from a bacterial plate.

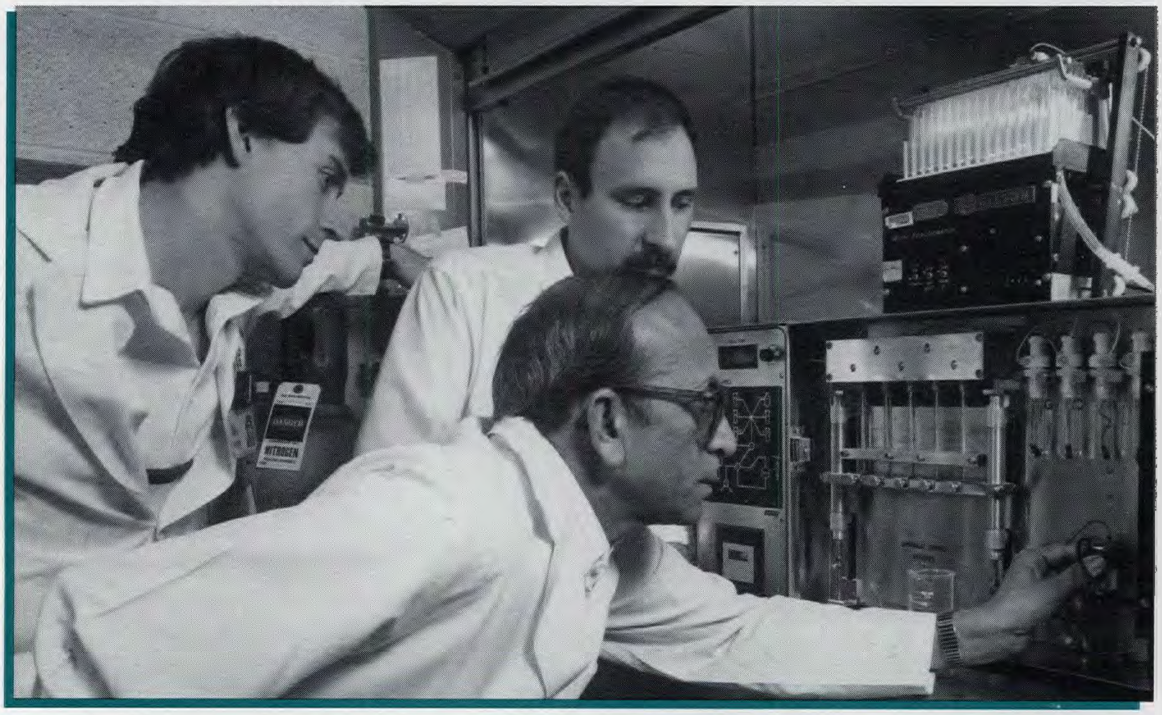
this amino acid substitution, we have cloned the gene for human EGF and altered the genetic code so that it calls for the manufacture of the desired mutant proteins.

Our initial task of synthesizing the human EGF gene (aided by an automated DNA synthesizer) and cloning it into the bacterium, *Escherichia coli*, was achieved with the help of graduate students David Engler and Risë Matsunami. We showed, by amino acid sequencing studies, that our cloned gene produces a protein identical to human EGF—in milligram quantities per liter of cells. Moreover, the cloned gene product is identical to authentic human EGF in its biological properties.

Our mutagenesis studies were targeted at specific amino acid residues in the EGF molecule, chosen on the basis of their species invariance and published physicochemical studies of EGF. Replacements of the amino acid residues were achieved by oligonucleotide primer–directed mutagenesis of the EGF gene. The oligonucleotide primers (so-called because they initiate, or prime, the synthesis of DNA) contained the code alterations needed to produce the desired mutations (amino acid substitutions). These code "insertions" then primed the synthesis of genes for mutant EGFs, which were subsequently expressed in the *E. coli*. Steve Campion, a biochemist from the University of Notre Dame who joined our group in late 1986 as a postdoctoral research associate, also contributed to our progress in the protein engineering of EGF by designing, producing, and characterizing some interesting EGF mutant proteins.



David Engler examines a flask of *Escherichia coli,* the bacteria used to produce the human EGF protein product from genes cloned in Niyogi's laboratory.



Salil Niyogi prepares the gene synthesizer for operation with help from graduate student David Engler (left) and Stephen Campion, a postdoctoral fellow.

By altering the genetic code for the cloned EGF gene to achieve amino acid substitutions in the protein growth factor product, we have identified the amino acid residues needed for EGF's function. Among the mutant proteins we have produced are two that bind to the EGF receptor but are unable to properly stimulate the tyrosine kinase growth-regulating activity. One of these EGF analogs is made by replacing the amino acid isoleucine with threonine at the 23rd position in loop B of the molecule (see figure on p. 54). The other binding mutant is produced by substituting glycine for leucine at the 26th position in the same loop of EGF. We believe these mutant proteins may even inhibit cell growth, making them potentially useful for cancer treatment.

We are testing these two analogs to determine the extent to which they can inhibit EGF-stimulated cell growth. They will also be tested to determine whether they can inhibit the activity of transforming growth factor alpha (TGF-α), which is structurally related to EGF and which also initiates its action by binding to the EGF receptor. TGF-α is found in all human tumors and cells transformed by RNA tumor viruses. An EGF analog that could act as an antagonist of both EGF and TGF-α might prove to be an important anticancer agent. It is also possible that this research could lead to a "super EGF" analog that might stimulate very rapid healing and greatly improve medical treatment of burns and severe wounds. ornl

Salil K. Niyogi, a native of Calcutta, India, who holds a Ph.D. degree in biochemistry from Northwestern University, is a senior research scientist in ORNL's Biology Division. He is a fellow of the American Association for the Advancement of Science and an adjunct professor at the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences.

Audrey Stevens is a senior research scientist in ORNL's Biology Division and an adjunct professor at the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences. A native of Leigh, Nebraska, with a Ph.D. degree in biochemistry from Case Western Reserve University, she is a recipient of the Outstanding Scientist Award from the state of Maryland and a fellow of the American Association for the Advancement of Science.

DNA Repair— A Recipe for Survival

Research is zeroing in on the cell's mechanisms for self-repair.

By Sankar Mitra

For some time it has been recognized that DNA, the universal genetic material that contains all information needed to maintain and reproduce living organisms, is not totally inert—chemically speaking. The two strands of DNA, intertwined in a double-helix structure, are held together by hydrogen bonds between its basic units, called bases. The four types of bases are paired uniquely—that is, between adenine (A) on one strand and thymine (T) on the other or between guanine (G) and cytosine (C). This cardinal rule of base-pairing allows the exact reconstruction of each DNA strand by its complementary strand during cellular multiplication (replication). The sequence of the four bases in each strand of DNA is unique for each organism and thus defines that organism.

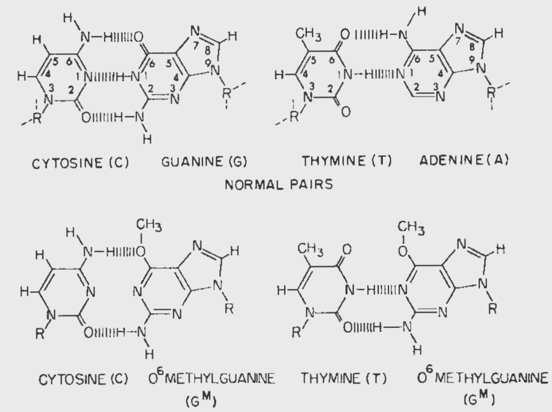
The integrity of DNA is damaged naturally by chemical alteration of cytosine and spontaneous base losses. In addition, DNA bases may be altered or destroyed by foreign agents, including ultraviolet and ionizing radiation and a variety of chemicals in the environment (e.g., fossil fuel by-products). The effects of these damaging agents include inhibition of DNA replication, which leads to cell death, or alteration of the sequences of DNA bases, which results in mutations, tumor induction, and cell death. Although mutations are thought to be responsible for the evolution and diversification of living organisms, genetic alterations cannot be too frequent, or such instability would lead to the extinction of species.

It turns out that all organisms have developed ways to repair DNA damage or to cope with it and continue to function. Furthermore, repair pathways are diverse, as might be expected from the several distinct types of alterations produced in DNA by a variety of agents.

Our interest in DNA repair is focused on the damage induced by alkylating carcinogens and mutagens. These chemicals include, for example, nitrosamines present in food or produced in the stomach, as well as industrial chemicals such as ethylene oxide (see Generoso's article on p. 24). Alkylation is a chemical process in which an alkyl radical (an ionized molecule containing carbon and hydrogen) is incorporated in an organic compound by substitution or addition. Among the various types of changes produced in DNA by these agents, alkylation at the oxygen attached to the 6th carbon position (O^6) of the base G has been shown to cause the most critical damage, leading to mutation and tumor induction. This result probably stems from the propensity of O^6-alkyl G to pair with T instead of its normal pairing with C (see figure at right). Such a mispairing leads to a mutation.

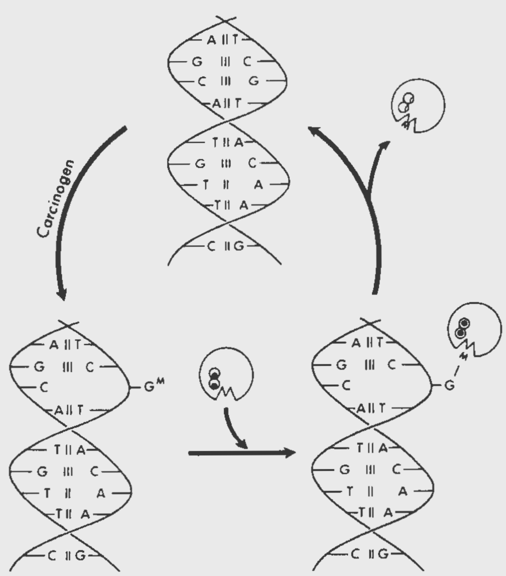
Sankar Mitra uses the "gene synthesis" machine for protein engineering.

Suicide Reaction Discovered



Base pairing in duplex DNA. Normal pairs are in the top row. Bottom row shows pairing of O^6-methylguanine.

In 1980, Robert S. Foote, Bimal C. Pal, and I (and an independent group in England) showed that the repair of this modified G (G^M) in DNA involves an unusual reaction never before seen. The process does not involve the mechanisms most commonly observed, which are (1) removal of the damaged base or a segment of DNA spanning the damaged site and (2) base replacement directed by the complementary strand. Instead, removal of the lesion involves a direct extraction of the modifying alkyl group such that the original G is restored without any effect on DNA. Shown as the "Pac-man" protein in the figure below, O^6-methylguanine-DNA methyltransferase (MT), accepts an alkyl group (on a specific cysteine residue) in a suicide reaction—the self-alkylation destroys (inactivates) the MT protein. This reaction is absolutely specific for O^6-alkylated G and is rather unique, because biochemical processes are typically catalyzed by enzymes that remain unchanged after the reaction. As the figure below illustrates, the DNA molecule may again receive an alkyl group by exposure to a carcinogen, and the cycle can repeat itself as long as the MT is available.



This figure illustrates an unusual DNA-repair reaction discovered in 1980.

The MT protein is present in almost all organisms, although the human MT is very distinct from that of the bacterium *Escherichia coli*, which is routinely used as the model laboratory microbe.

The level of MT is highly regulated in both mammals and bacteria. The *E. coli*–MT, which is also called the Ada protein, plays a central role in its own regulation—controlling the activity of its gene by binding, in a modified form, to the regulatory sequence. The recently available recombinant clone containing the *E. coli*–MT gene was an attractive candidate for our site-directed mutagenesis research designed to unravel the complex and multiple reaction modes of this protein.

Normal and Mutant Repair Proteins

Our first objective was to produce the *E. coli*–MT protein in quantities large enough to allow definition of its biochemical characteristics. We suspected that the observed lack of high yield of the protein from a recombinant vector was attributable to the negative function of the regulatory sequence. Our hunch was proved to be correct when Keizo Tano (university guest from Japan), Foote, and I applied a site-directed mutagenesis approach to delete the DNA segment identified as the regulatory region. In the modified recombinant, the MT protein represents 10 to 15% of the total protein harboring the altered gene. Debasish Bhattacharyya, a university guest from India in our group, then showed that this protein can be prepared very rapidly in a nearly pure, completely active form. A number of laboratories in the United States and the United Kingdom are planning to use our system for its large-scale preparation.

To learn more about the relationship between the structure and function of the Ada protein, we performed site-directed mutagenesis on the specific cysteine residue (Cys_{321}) that was shown earlier to be the alkyl acceptor site. We created two mutant proteins: one (mutant I) was made by substituting histidine for Cys_{321} and the other (mutant II) by inverting the normal $Cys_{321}His_{322}$ sequence (i.e., to $His_{321}Cys_{322}$). Our reason for constructing such mutants was that both cysteine and histidine could, in principle, function as alkyl acceptors and that the normal $Cys_{321}His_{322}$ sequence is conserved in other enzymes involved in different types of methyl transfer reaction.

We were surprised to find that, under the standard conditions used for inducing normal Ada protein synthesis, mutant I was not produced at all. Although mutant II was produced at a low level, it was smaller in size than the wild-type (normal) protein.

Further investigation showed that the subtle changes we induced in the mutant proteins caused a major perturbation in their regulatory role and their stability inside the cell. The synthesis of mutant I in the host bacteria was inhibited because the mutant protein acted as a negative regulator, in contrast to the positive regulatory role of the normal Ada protein. Both mutant I and mutant II proteins could be produced by appropriate genetic manipulation under unusual conditions. However, these proteins, unlike the normal protein, were extremely sensitive to degradation in the cell. Finally, neither of the mutant proteins could accept the alkyl group from the O^6-alkylated guanine.

These results illustrate that a subtle change in a protein can result in a profound change in both biological and chemical activities. We are currently creating additional mutants of this protein in the region of its putative DNA-binding sequence. Experiments with these mutants should elucidate the molecular mechanism that activates the alkylation-damage repair by the Ada protein.

At the same time, our collaborative effort to determine the three-dimensional structure of the Ada protein by X-ray crystallography may provide information about its overall geometry that could enable us to create site-directed mutants having broader, or more desirable, specificity in repair of DNA damages. We are also attempting to clone a recombinant vector from which the human MT protein can be expressed. The availability of such a clone would open up the exciting prospect of creating human MT having more desirable reaction specificity and understanding how our body regulates the repair activity. ornl

Sankar Mitra, a native of Calcutta, India, who holds a Ph.D. degree in biochemistry from the University of Wisconsin, is leader of the Nucleic Acid–Protein Transaction Group in ORNL's Biology Division. He is also an adjunct professor at the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences.

Mitra and William Dunn watch as Julia Collier, an ORAU trainee, injects a sample for high-performance liquid chromatographic analysis.

Can the CO_2 Fixation Enzyme Be Improved?

By Fred C. Hartman

Every day the world's most abundant protein sets off chemical reactions that convert an atmospheric gas into plant food, which is essential to human survival. The protein, however, could do its work more efficiently and possibly increase food crop yields if it were redesigned. At ORNL efforts are being made to alter this protein appropriately by state-of-the-art "protein engineering" techniques.

Photosynthetic bacteria and plants use the electromagnetic energy of sunlight that is trapped by their chlorophyll–protein complexes to drive the biosynthesis of carbohydrates from atmospheric carbon dioxide. About 10^{11} tons of carbon are converted into organic compounds annually by photosynthesis. Although not generally appreciated, more than half of the ongoing photosynthesis in the biosphere is carried out by microscopic organisms in the oceans.

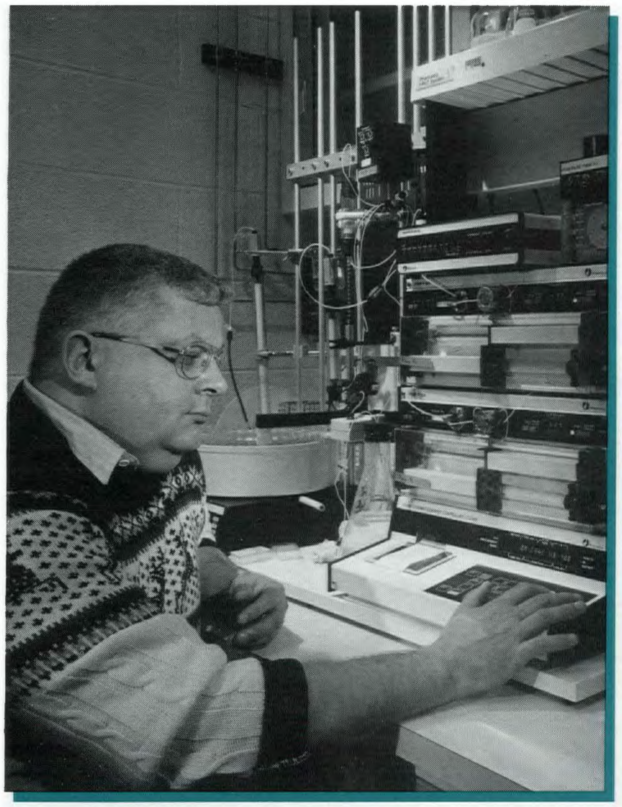
Fred Hartman (standing) and Richard Mural examine a computer model of the "Rubisco" enzyme, which appears in color on the front cover. Understanding the structure-function relationships of this carbon-fixing protein could lead to increased production of biomass for food and energy.

Like all complex biosynthetic pathways, the conversion of carbon dioxide into carbohydrates is a multistep process in which each discrete chemical reaction is catalyzed by a unique enzyme (type of protein). The initial reaction in this pathway (known as the Calvin cycle) is the carbon dioxide fixation reaction, which is catalyzed by ribulose-bisphosphate carboxylase/oxygenase, commonly called Rubisco. This enzyme links the inorganic and organic carbon cycles of the biosphere by catalyzing the only known reaction through which atmospheric carbon dioxide can sustain living organisms.

Most Abundant Protein

Carbon dioxide fixation is rather inefficient and severely constrains the overall rate of photosynthesis. For Rubisco, the catalytic constant (a conventional measure of maximum catalytic rate) is only 2 to 5/s, whereas for the vast majority of enzymes the value is 10^3 to 10^5/s. Photosynthetic organisms compensate for this gross inefficiency by synthesizing huge amounts of Rubisco, frequently accounting for >50% of the organism's total soluble protein. Thus, Rubisco has the dubious distinction of being the world's most abundant protein. The organism pays a huge price in its energy economy for committing so much of its nitrogen nutrient to this one enzyme to catalyze one chemical reaction.

To make matters worse, Rubisco also catalyzes a competing reaction of



Tom Soper uses high-performance liquid chromatography to rapidly isolate mutationally altered proteins.

oxygen with ribulose-bisphosphate, an energy-wasteful degradative reaction having no known physiological function. Although the relative specificity of Rubisco favors carbon dioxide fixation, the prevalence of oxygen in the atmosphere diminishes the rates of photosynthesis. The competitive effects of the oxygen reaction can cut yields of many food crops in half.

Theoretically, plant yields could be enhanced by increasing Rubisco's catalytic efficiency for photosynthesis and/or its specificity for carbon dioxide fixation. Laboratories throughout the world have reached a consensus that the feasibility of engineering a more efficient Rubisco requires more information about the structure and mechanisms of the enzyme. Our group at ORNL uses protein engineering to systematically replace amino acids with others at the catalytic center of Rubisco to determine which ones play key roles in the enzyme's function. Such structure-function correlations may offer new insights for improving Rubisco.

Mutant Rubisco Genes

The local team that is trying to unravel the mysteries of Rubisco includes Bob Foote, an organic chemist who synthesizes fragments of DNA that are used to insert the programmed mutations into the Rubisco gene; Frank Larimer and Richard Mural, molecular geneticists who place the mutated genes into simple bacteria, which then serve as miniature factories for producing the new forms of Rubisco; and Tom Soper, Claude Stringer, and myself, protein chemists who isolate and characterize these new forms of Rubisco. Eva Lee, a postdoctoral investigator, and Harry Smith, a graduate student, also evaluate the properties of the mutant enzymes.

One of the greatest challenges in protein engineering is the design rationale for structural alterations. The Rubisco molecule is 475 amino acids long; each specific amino acid at each of the 475 positions could be replaced by any of the other 19 constituent amino acids. Hence, to survey the effects of all possible single amino acid substitutions would require the construction of 9025 different mutant proteins! Construction, isolation, and characterization of just one mutant protein can require many months of work, so very judicious choices must be made.

Fortunately, our previous work has pinpointed several amino acid loci in Rubisco where reactants bind and catalysis is achieved. This catalytic center of the enzyme is the focus of our research. To date, we have prepared several dozen new forms of Rubisco having altered catalytic centers. Evaluating their properties has allowed us to identify the amino acid that functions as the acid/base catalyst to initiate the complex, multistep carbon dioxide fixation reaction and to identify another amino acid that stabilizes an intermediate in the reaction pathway. This progress encourages us toward our ultimate goals of designing a gene that expresses a superior Rubisco and placing that gene into plants, thereby transforming them into species having improved photosynthetic (thus, food-producing) efficiencies. ornl

Evolution of ORNL's Environmental Sciences

By Stanley I. Auerbach

Evolution in the biological sense refers to an essentially random process involving the natural selection of those attributes that provide optimal conditions for growth and maintenance of a population of organisms. In some respects, the history of the development of environmental sciences at Oak Ridge National Laboratory can be viewed as being somewhat analogous to an evolutionary process. Consider the following statements:

- "This . . . interim survey of concentration and release of radionuclides by aquatic organisms . . . of immediate benefit to ORNL in the disposal of liquid wastes . . . also represents the start of a five-year program designed to establish safe and economical methods of disposing of liquid wastes associated with power reactors."—Karl Z. Morgan, January 1954.
- "This, or higher factors, may obtain for certain links in the complicated ecological web, but perhaps these links can be wiped out, and the ecological universe will have the capacity to readjust itself and reach a new type of equilibrium that will satisfactorily meet the requirements of man and his environment." Summary of comments by I. I. Rabi, K. Z. Morgan, A. M. Weinberg, and O. Park, at a meeting in the Health Physics Division, September 1954.
- "To date, as shown by the efforts of the survey of 1950 to 1953 and continuing observations by Oak Ridge National Laboratory, no catastrophic effects have occurred to the fauna and flora due to radioactive waste effluent and the accompanying continuous 12 years of exposure."—Charles S. Shoup (AEC-ORO), 1955.
- "We appreciate the interest of the Laboratory in proposing the additional ecological studies, but in view of the current plans of the Division of Biology and Medicine, it is suggested that budgetary provision for the additional work be deleted from the budget submission for FY 1956."—Samuel R. Sapirie, February 1955.
- "It seems essential that an ecology program be so organized as to utilize techniques and modes of thought peculiar to the point of view. It thus may contribute to the needs of Health Physics rather than attempt to meet those needs by way of a partial approach. Ecology deals with whole organisms in whole communities under whole environments. If an ecological program on each of the various sites . . . seems desirable, it is suggested that it be set up to meet its own objectives."—John N. Wolfe, May 1956.
- "We believe that the ecology program at ORNL should be based on the waste disposal problem on the following considerations. . . . The problem of radioactive waste disposal is certainly one of the most abiding deterrents to economical nuclear power . . . . If, in the view of the Commission, this minimum effort . . . (6.5 man-years) . . . cannot be achieved in due course, the recommendation of the Laboratory is that responsibility for exploration of this vitally important area of potential hazard be

Stan Auerbach in front of the Environmental Sciences Building at ORNL.

transferred elsewhere"—John A. Swartout, July 1956.

- "In 20 years, I would envisage the Oak Ridge Reservation studded with field experiments employing radioisotopes . . . with complementary experiments simultaneously under way in different regions and countries. A permanent staff of several hundred environmental scientists would also use laboratories with large-scale facilities . . . such a program could draw upon and contribute to an international graduate educational center as well as become an international information exchange."—Stanley I. Auerbach, February 1962.

These contrasting views and recommendations reflect more than the usual struggles associated with the establishment of a new program. A new area of science was being promulgated; one whose ideas, concepts, and needs were associated directly with a perceived national problem. Conceptually, ecology was starting to be visualized as an area of science whose time for application to a totally new social problem had come. The early struggles reflected many things—lack of understanding of the limitations of the science, the importance of defining the field in the context of national laboratory responsibilities, and the urgent need to shift from observational science to a more quantitative and, therefore, more rigorous understanding of ecological processes.

Early Monitoring

Shortly after the establishment of the Clinton Laboratories (ORNL) in 1942, it became necessary to dispose of radioactive by-products from the various operations. Based on geological considerations, Melton Valley–White Oak Creek and White Oak Lake were selected for disposal sites and for radioactive effluent releases. The need for protective criteria and surveillance was recognized early by Karl Morgan, the pioneering health physicist and founding director of the Health Physics Division.

By 1943, routine monitoring and surveillance for various radionuclides, including transuranics and fission products, was being carried out in the White Oak Creek–White Oak Lake area, which served as the regulating point for the controlled release of these radionuclides to the Clinch River.

By the late 1940s, radionuclides had built up in the holding basin and drainage system enough that an ecological survey of area fauna and flora was

Trees were tagged with cesium-137 in this first experiment using radioactive isotopes to study forest system processes (May 1962). The individuals involved were, from left, W. C. Cate, J. S. Olsen, H. D. Waller, and S. I. Auerbach.

Gladys Dodson and Marvin Shanks checked leaf decomposition and isotope release rates in an early ecology study headed by Dave Reichle.

suggested to evaluate the impacts of the radioactivity. In response to this need, the Atomic Energy Commission (AEC) authorized the Laboratory to enter into an agreement with the Tennessee Valley Authority (TVA) to carry out such a survey. Fish studies were done in 1948 to 1949, and the main survey followed from 1950 to 1953 under the leadership of the late Louis Krumholz, a well-known aquatic ecologist. The survey findings stirred some controversy, but, in general, the releases were not linked to any serious problems.

By that time, interest had intensified in the perceived problem of nuclear-power–related radioactive waste. A new waste disposal program that included ecological research was established in the Health Physics Division under Edward G. Struxness. A well-known American ecologist, Orlando Park of Northwestern University, helped plan the new program with the initial purpose of determining the impacts of radioactive wastes on the environment through a series of laboratory experiments. Funding was made available in 1954, and I was employed to carry out these investigations and lay the basis for an expanded, longer-term effort.

As a result of work here and elsewhere, the AEC set up a national ecology program headquartered in Washington. John N. Wolfe, a well-known ecologist, was appointed its director. Wolfe thought field studies should receive more emphasis and encouraged ORNL to shift its focus accordingly. The draining of White Oak Lake in 1955 provided a unique, radioactively contaminated habitat for conducting ecological research during the next several years. The draining of White Oak Lake also resulted in a large instant release of radioactive fission products to the Clinch River, particularly radioactive cesium, cobalt, and strontium. A unique, multiagency effort was organized by Struxness to determine whether radiological hazards existed as a result in the lower Clinch River and in portions of the Tennessee River. To meet the needs of this new effort, an aquatic component under Daniel Nelson was added to the ecology program. During these years (1956 to 1961) our work was relocated to the Oak Ridge Y-12 Plant because of a space shortage at ORNL.

During this time, ORNL Director Alvin M. Weinberg maintained a strong and encouraging interest in both the program and the field of ecology. The work on the lake bed provided new in-

The Office of Civil Defense sponsored this study of post-nuclear-attack ecology in 1967. To study the effects of fallout, a simulated, highly radioactive, nuclear fallout material was dispersed over several small plots using this remotely controlled spreading device.

sights into problems associated with whole-habitat contamination and its effects on mammals, birds, insects, and plants. It became clear to the ecology staff that a study of the long-term environmental problems associated with radioactive waste disposal required new field-based experiments, using known quantities of radionuclides. Data from such studies would enable ecologists to predict the behavior of these substances in ecological systems.

The early 1960s were a dynamic period for the program, bringing expansion of the Y-12 Plant facilities, establishment of an ecology library, and increasing worldwide attention to the environmental problems associated with nuclear energy. Because of the latter, the ecology staff began to focus on developing increasingly larger-scale field experiments that involved total-systems approaches.

Meanwhile, planning for future growth of the program intensified. The completion of an addition to the main research building complex at ORNL made available the Health Physics Division building (Building 2001) for conversion to an ecology laboratory. This was a period of dynamic planning and vision at ORNL, and the ecologists were encouraged to take an active part. "Where would ecology be 20 years in the future?" was a challenge laid down by the Laboratory Director, and to which we responded. Such futuristic thinking prompted the planning of a totally new facility designed especially for large-scale ecological and related environmental research. I proposed what was then a radically new design, namely a circular configuration for the main building, later jokingly referred to as the ecology "wedding cake."

Ecosystem Studies

Following the move back to ORNL, plans were made to initiate a unique forest field experiment that would involve tagging an entire forest stand with radioactive cesium—a major fission product and component of radioactive fallout. This experiment began in 1962. A whole series of smaller, associated experiments under the leadership of David E. Reichle later used radioisotopes to clarify key subprocesses in the forest nutrient cycle and enabled ecologists to quantify them for the first time. The experiments determined the roles of bacteria and insects in the decomposition of

Stan Auerbach is shown testing the amphibious, all-terrain "Wolverine," one of several unusual vehicles used by ESD for conducting ecology field studies.

organic matter on the forest floor and the rates of nutrient cycling in specific soil types and related investigations. These projects attracted national attention to ORNL as a center for advanced studies in ecosystems and led to another major development in the ORNL environmental efforts—the application of systems analysis methods to the study of both basic and applied ecological processes. New mathematical tools also offered the possibility of developing computer models to simulate ecosystems, thus enabling the investigator to predict the future behavior of radioelements in environmental situations (e.g., their passage through the ecological food chain.)

Ecology Education

ORNL also has evolved as a center for ecological education. In conjunction with Oak Ridge Associated Universities (ORAU), a series of summer institutes in radioecology was established in 1963 to train scientists and educators in the new techniques and ideas being developed at ORNL. A one-year advanced course in the totally new (and ORNL-conceived) field of systems ecology was proposed to the University of Tennessee (UT) and taught in 1965 at UT by three members of our ecology program (Jerry Olson, Bernard Patten, and George Van Dyne), each of whom became internationally known leaders in systems ecology. ORNL-UT interactions, which have existed since the first years of the program, have intensified over the years.

University faculty and ORNL ecology staff also worked together in setting up a new graduate program in ecology in 1967 at UT. The new graduate program not only educated students in this new field but provided opportunities for field experience and graduate research at ORNL. During the two decades since the program was established, scores of students (both graduate and undergraduate) have participated or earned degrees in ecological studies at ORNL. Other educational ventures sponsored by our ecological studies include special honors programs at several universities and colleges to bring advanced undergraduates to ORNL for a term of research and summer study opportunities for outstanding high school students.

Worldwide Environmental Concern

In the mid-1960s, environmental concerns emerged on a national and international scale. Worldwide radioactive fallout contamination of the food chain had a major role in developing this awareness. Pollution of lakes, streams, and rivers also began to attract public and, hence, political attention. Members of the international ecological research community increasingly expressed concern over growing damage to the planet's environment; they called for new research programs that would assess the state of the earth's ecosystems and provide a scientific basis for predicting the future consequences of human activities on the biosphere.

This original ecology workboat, which was designed and built in 1959 for the Clinch River Survey, was used in aquatic investigations for nearly 20 years. Collecting specimens are, from left, Bill Martin (now with Tennessee Tech), Neal Griffin, and Dan Nelson (deceased).

A major new international program was promulgated by the world's leading ecologists—the International Biological Program (IBP). In the United States, IBP received a special appropriation from the Congress in 1967 and became the responsibility of the National Science Foundation (NSF). Soon organized under the National Academy of Sciences, it came to be known as the Analysis of Ecosystems Program. The research was organized according to major U.S. ecological regions (biomes); within each region, large teams of ecologists and other environmental scientists were selected to carry out integrated studies on the major ecosystems. Hundreds of scientists and graduate students became involved in this effort and, because of its established reputation, the ecology group at ORNL was asked by the NSF to lead the program for the eastern forest region—a major role (see article by Elwood, et al. on p. 94). This was the first time an atomic energy laboratory received NSF funding to carry out a major program. The funding begun in 1968, which provided the means for a major expansion of the ORNL program, and started a relationship with the NSF that has continued well beyond the end of the IBP in 1976.

During this same period (1965 to 1976), other activities and events occurring at ORNL and in the ecology program affected the development of environmental sciences. Once the integrated study of the Clinch River was completed and judged to be a successful one-of-a-kind endeavor, ORNL aquatic ecologists turned their sights toward the Oak Ridge Reservation. Although White Oak Lake had been drained, the construction of Melton Hill Dam on the Clinch River necessitated the impounding of a small portion of the lake for radiological control purposes. The populations of fish and insects in this impoundment provided an opportunity to study the long-term genetic effects of radioactivity on organisms. Integrated research projects dealing with the long-term impact of radioactivity on whole ecosystems were very much on the minds of ORNL ecologists at this time, and two major projects became our goals: (1) to define and model the processes of an entire

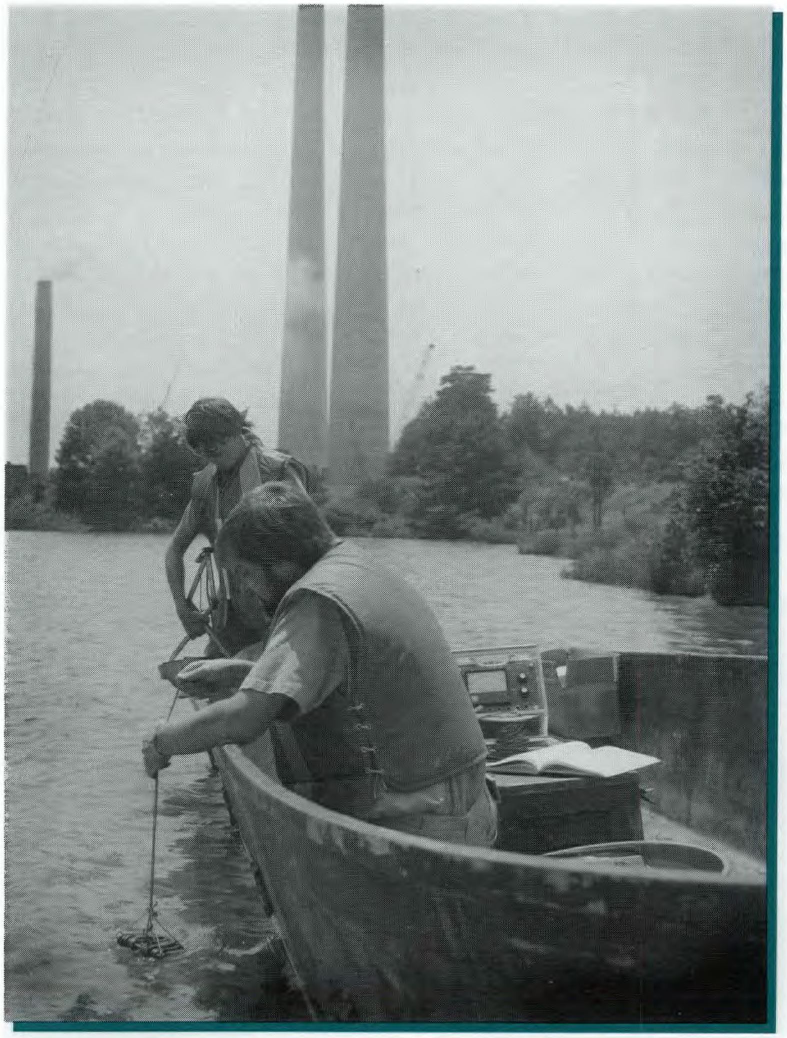
Gordon Blaylock and Neal Griffin are shown collecting mosquitofish from contaminated White Oak Lake (1968) for studies of the effects of low-level radiation on fish population fecundity.

watershed system and (2) to study the long-term consequences of radioactive fallout on an agricultural-type system.

A suitable site on the Oak Ridge Reservation was chosen for the watershed study—the Walker Branch Watershed located 3 miles east of ORNL. The proposed research program was approved by the AEC, which funded the project. Construction of the small dams (weirs) necessary to the project was started in 1967. Thus began, under Daniel J. Nelson and James W. Curlin, a keystone project in environmental sciences at ORNL. During the next 20 years this project provided not only fundamental knowledge about processes in the landscape but also information that proved applicable in local waste management issues.

A field experiment led by Paul B. Dunaway to study the effects of radioactive fallout on agricultural systems was proposed to be performed on ~10 acres of formerly river-bottom agricultural land west of the Laboratory. The pine plantations planted as part of an ill-conceived AEC forestry effort 10 years earlier were cut and the field seeded to pasture. Although this proposed project was not approved, the site, now known as the 0800 Experimental Area, became a key site for a variety of environmental investigations of the behavior of radionuclides and other potentially hazardous substances, among which was a special study of the ecological effects of nuclear war, sponsored by the Office of Civil Defense. This was a much smaller-scale version of the project initially proposed to AEC. In recent years, the area has been the location of a major air pollution and acid rain experiment—one of the largest of its kind anywhere.

As the decade of the 1960s waned, public concern over environmental problems increased, and the resulting ferment at ORNL produced several new environmental programs. Nuclear power came under greater attack, and the staff became involved in these controversies. An emerging issue in the power controversy was the potential impact of releasing the water heated by reactor cooling systems. Few or no data existed on the effects of heated waters on aquatic flora and



As part of ESD's Power Plant Thermal Effects Project (1971 to 1977), Lynn Wright (back) and Jack Mattice (now with EPRI) collected aquatic organisms near the cooling water intakes of TVA's Kingston Steam Plant, a coal-fired electric generating station.

fauna, and a new aquatic research project, to be led by Chuck Coutant, was approved by the AEC. The funds made available included support for the construction of a new, computerized test facility in the early 1970s, which became the forerunner of the Aquatic Ecology Laboratory.

A New Division

Programs in regional studies, environmental quality, and socioeconomic issues associated with environmental problems became more active at the Laboratory during the early 1970s. An in-house training program in environmental sciences for professional staff was also developed.

By 1970, the Laboratory had an ecological science research unit that was recognized internationally for its contributions and leadership. Although there were other environmental components in the Laboratory, none had achieved either the magnitude or the distinction of the ecology unit, and in March 1970 the unit was elevated to division status—the Ecological Sciences Division.

The Division's creation brought more challenges. Although ecology was to play a pivotal role, many other opportunities and concerns existed, both local and national, that involved other environmental science disciplines. Two events in the new decade helped to broaden our focus and ORNL's programs in this area.

In 1970, the National Environmental Policy Act was enacted, requiring agencies to prepare statements on the possible environmental impacts of any major proposed action. Although at first somewhat ignored, a judgment in a court case involving the licensing of a proposed nuclear power plant at Calvert Cliffs, Maryland, galvanized the entire impact process. Environmental impact statements were mandated for nuclear power plants, so the AEC directed its national laboratories having large resident environmental programs to create special units to assist in the preparation of these statements. At ORNL, this responsibility fell to the Ecological Sciences Division. Within months a large (50- to 60-person) and well-organized effort was under way, and staff members were retrained in the new field of environmental impact assessment, emphasizing the connection between research and interpretation.

The second pivotal event of the 1970s for ORNL's environmental sciences programs was the reorganization of the Health Physics Division. Its environmental components were shifted to other laboratory organizations, and the radiological and environmental assessment and modeling group, as well as several geologists who had been part of a waste disposal research group, joined the Ecological Sciences Division. The broadening dimensions of the division were reflected in its renaming, in April 1972, to the Environmental Sciences Division (ESD).

Within a year after approval of the broadened scope, an appropriation for a new environmental sciences building was forthcoming. Key staff members participated in site selection and design, and groundbreaking took place in 1975.

A wide spectrum of local environmental problems began to be addressed, including local low-level radioactive waste burial grounds and the chemical pollution of reservation streams. Although these concerns were evident to the environmental scientists in the division, almost a decade elapsed before significant action was taken to address problems in these areas.

The nationwide shift from interest in nuclear-related problems to those associated with fossil fuels also started in 1975. The environmental sciences again played a major role in helping ORNL shift its research emphasis to the new programs. The environmental questions associated with coal created significant new research challenges—global warming, acid rain, and toxic synthetic compounds—and ESD staff members are leaders in these investigations. The occupancy of the new environmental sciences building in 1978 signaled another step in the evolutionary process begun three decades earlier.

Whole-body counts were done on fish in this 1963 study to determine rates of elimination and biological half-lives of radionuclides in live animals.

The Controversial 1980s

In the early 1980s, ESD became involved in a number of controversial issues, all of which had their origins in events or activities that occurred much earlier.

Environmental activists in the 1960s began to openly question the presence of power plants near various water bodies. Tensions continued to increase, and in the 1970s a controversy arose in the Hudson River Valley over the proposed construction of a nuclear power plant whose cooling systems might pose a threat to the fisheries of the river and Atlantic coast. ESD aquatic scientists became involved because of the environmental impact statement they had prepared in 1972 on the proposed plant. During the next decade of continuing judicial hearings, we helped resolve this complex scientific and legal controversy that is a landmark in applied aquatic ecology.

In 1982 and 1983, the revelation of significant mercury spills made in the mid-1950s into local streams from the Oak Ridge Y-12 Plant provoked a public controversy. ESD scientists had noted high mercury levels during their 1970 stream studies, and this information had been brought periodically to the attention of my management. ESD scientists had done extensive mercury studies around the famous mercury mines in Spain and in mercury-contaminated rivers and lakes in the southeastern United States. Ironically, the division's expertise and established reputation in analyzing the environmental behavior of mercury was not used to address the local mercury problem at that time. Since 1983, however, ESD has been responsible for a long-term biomonitoring effort to evaluate mercury contamination hazards and the results of remedial actions on the Oak Ridge Reservation.

Another major contribution in the 1980s was a detailed analysis, by ESD's John Trabalka, of an alleged major nuclear accident in the Soviet Union, said to have occurred in 1957. Trabalka inferred details about the "accident" by analyzing Russian technical papers on intriguing radioecology studies from the late 1950s through the 1970s. Because the accident was alleged to be related to radioactive waste, a certain public notoriety developed about this event, especially since the Soviet government denied its occurrence. The CBS television program "60 Minutes" became interested, and my office was the site of an interview involving Dan Rather (now the "CBS Evening News" anchorman), Trabalka, and me. A number of skeptics questioned our analysis, so we were pleased to learn recently that a top Soviet nuclear power official finally confirmed (in December 1988) that there was indeed a serious nuclear accident in the Ural Mountains 31 years ago.

In many ways, the occupancy of the new environmental sciences building marked both a culmination and a new beginning. Our currently preeminent role in the global carbon cycle work stems from our ecosystem studies of the previous decade. Likewise our national leadership in research on renewable resources for energy (biomass) is a natural extension of our years of research and leadership in forest ecosystem ecology. The earth sciences, which had been almost nonexistent at ORNL, were vigorously promoted by ESD activities such as our geological and hydrogeological studies.

Public concern over environmental and ecological problems is intensifying, both nationally and globally. The decades ahead will offer many challenges and opportunities to the new ESD leadership. Building on our strengths and past accomplishments, ORNL is well-positioned to continue world-class research in many of these challenging areas. ornl

Stanley I. Auerbach was director of ORNL's Environmental Sciences Division from 1970 through 1986. He holds a Ph.D. in ecology from Northwestern University and taught radiation ecology at the universities of Georgia and Tennessee. He is a fellow of the American Association for the Advancement of Science, former president of the Ecological Society of America, and former director of the Eastern Deciduous Forest Biome Project of the International Biome Program. He is recipient of the Leadership Award of the U.S. International Biological Program, the Leadership and Service Award of the Ecological Society of America, and the Distinguished Associate Award of the U.S. Department of Energy.

ESD's Educational Impacts

By David E. Reichle and Judy L. Trimble

In the current era of concern over increasing this nation's industrial competitiveness, much attention at the national laboratories is being devoted to technology transfer. A complementary activity is providing the next generation of scientists with the training and experience needed to help our nation develop new technology that is both economically competitive and environmentally acceptable.

The Environmental Sciences Division (ESD) at ORNL is playing an active role in this leadership training, based on our long-standing tradition of close academic association extending over four decades. Our contributions encompass several areas:

- Research subcontracting with academic institutions for national research and development (R&D) programs to focus academic expertise on national problems;
- Research collaboration of ESD professional staff with experts from academic institutions to attack complex R&D problems;
- Internships and field experience for graduate students, postdoctoral fellows, and professionals on sabbatical leave, who are given opportunities to work on environmental science research projects in support of Division staff;
- Environmental awareness and career development for elementary and high school teachers and students and undergraduate science majors, who are invited to observe and participate in scientific research and to learn more about complex environmental issues.

ESD has long been recognized as a national center of excellence in ecosystem analysis and theoretical ecology, particularly in aquatic and terrestrial environmental toxicology, earth sciences related to hazardous waste disposal, landscape biogeochemistry, hydrology, and the development of quantitative techniques for environmental assessment. The Division's programs in acid deposition, biomass energy development, and global carbon dioxide research are internationally recognized.

Research Subcontracting

During FY 1988, ESD subcontracted nearly $11 million of research monies to 57 different universities and colleges, private institutions, and other federal organizations. Sixty-one percent went directly to universities. Currently, the Division has formal relationships with eight minority educational institutions, and Dean Sheard and Virginia Tolbert, ESD's Affirmative Action representatives, are actively pursuing collaborative efforts with others. The major subcontracting areas are in biomass production, global carbon dioxide, waste management R&D, and acid deposition research.

Training and Collaboration

In the late 1960s, ESD worked with the University of Tennessee (UT) to develop their Graduate Program in Ecology and, more recently, with UT's Waste Management Research and Education Institute (a Center of Excellence). Since 1973, 56 UT students who were supported by ESD in their graduate research have received degrees. Today, 21 ESD staff members have adjunct UT professorships in the program and 8 others have appointments at UT with the College of Forestry, Fisheries and Wildlife; the Oak Ridge Graduate School of Biomedical Sciences; the Plant Physiology and Genetics Program; and the Geography, Geological Sciences, Soil and Plant Sciences, and Zoology departments. Additional staff members have formal adjunct appointments with eight other universities: Tennessee Technological University, Vanderbilt University, Virginia Polytechnic Institute and State University, and the universities of Georgia, Illinois, North Carolina, Puerto Rico, and Göttingen in the Federal Republic of Germany

During the past year, ESD staff members served on 58 academic advisory committees, and 93 students and teachers representing 53 different

ESD educational activities benefit more than 100 precollege, college, and post-graduate students, 250 guest researchers, and 1500 visitors annually.

institutions were involved in various on-site educational programs at ORNL through appointments lasting three months or longer. ESD education coordinators Steve Herbes, Carolyn Hunsaker, and Pat Parr are assisting 34 graduate students and 20 postdoctoral fellows who are currently doing collaborative research in the Division. Sixteen of our graduate students and interns are from universities other than UT.

The Division's affirmative action role in education is noteworthy. During the past summer, 25% of our summer undergraduate students were minorities and over 50% of the current graduate students are women. Braulio Jimenez, ESD aquatic ecologist, has established an excellent educational liaison with the University of Puerto Rico, which sent six students to participate in our 1988 summer research opportunities.

Precollege Education

In 1988, ORNL-ESD was selected by the Department of Energy to host the first DOE High School Honors Program in the environmental sciences (joining programs in supercomputers, particle physics, superconductivity, life sciences, and synchrotron radiation research at other national laboratories). Gregg Marland and Bob Cushman of ESD worked with Linda Cain of the Office of University and Educational Programs to coordinate this program permitting 57 outstanding students (competitively selected from each state, 2 territories, and 5 foreign countries) to spend an intensive two weeks in ESD's research laboratories and in field studies. The students learned how applied environmental R&D is addressing problems such as waste management, water pollution, and climate change. These 57 students were in addition to the approximately 250 guests from academia, government, and industry who participate in division research each year.

DOE's 5500-ha Oak Ridge National Environmental Research Park is ESD's main outdoor research laboratory and is one of 13 user facilities at ORNL. More than 130 scientists annually take part in long-term research in the park, and

Leslie Henderson (right), an ESD intern and Roane State Community College student, discusses career opportunities with Judy Trimble and David Reichle, ESD Director.

university participants constitute the largest single user group. Park manager Pat Parr coordinates the user activities, including the Ecological and Physical Sciences Study Center's 10 field-study units designed for elementary, high school, and adult levels. Several units have been adapted for handicapped individuals.

The research facilities of the Division are also extensively visited by students. In FY 1988, Fred Baes, ESD tour coordinator, provided tours for more than 1500 visitors; the majority were from schools and colleges.

ESD does not build complicated technological prototypes nor typically patent new hardware developments. Our main emphasis is on scientific research and demonstration—the discovery of new knowledge that can be applied in resolving our planet's complex environmental issues. Much of this research is long term and provides a foundation on which sensible regulatory standards can be established. Scientific realism and an energy industry that considers environmental protection a necessary and cost-effective objective are our ultimate goals. Helping to develop a generation of scientists who understand how to advance the nation's scientific knowledge and technological progress while guarding the environment is the motivation for our educational activities. ornl

David E. Reichle is director of ORNL's Environmental Sciences Division. He joined ORNL in 1966, two years after earning his Ph.D. degree in biological sciences from Northwestern University. He is a recipient of the International Scientific Achievement Award of the Union of Forest Research Organizations and editor of three books: *Dynamic Properties of Forest Ecosystems*, *Analysis of Forest Ecosystems*, and *The Changing Carbon Cycle: A Global Analysis*.

Judy L. Trimble is manager of ESD's Technical and Administrative Support Organization. She previously served as technical assistant to Chester R. Richmond, ORNL's associate director for the Biomedical and Environmental Sciences. She holds an M.S. degree in plant pathology from Virginia Polytechnic Institute and State University.

A Puerto Rican student learns how to interpret and record ecological findings during her study at ESD.

Precollege Programs Thrive at ORNL

By Linda Cain

A little girl peers through a microscope and suddenly a drop of water comes alive. Boys scurry to collect rocks along their walk down a valley and, for them, the geology of East Tennessee becomes interesting and exciting. A high school group tours the Environmental Sciences Division's controlled environment facilities and suddenly the connections between acid rain, dying trees, and basic chemical concepts become clear and meaningful. These are some of the experiences that happen nearly every day at ORNL, as part of our precollege educational activities.

Many in government circles are concerned about our nation's growing "scientific illiteracy." Not only students, but a growing number of teachers, managers, and administrators seem to have little knowledge—and little curiosity—about science and how it affects the world. In addition, few of the "best and brightest" of our youngsters are choosing scientific careers. There is concern that, by the next century, we may not have enough scientists and engineers to meet our nation's technological needs.

Recognizing that science and technology are the underpinnings to our economy and the basis of our competitiveness in the world market, DOE has encouraged the national laboratories to share their scientific knowledge and facilities with the surrounding communities through a variety of educational programs. These activities are handled at ORNL through the University and Educational Programs Office, headed by David Rupert. There are many new happenings in education at ORNL these days, and some of the most exciting are in the precollege programs.

Study Center Activities

Our goals are clear: we want to encourage more students to choose careers in science and mathematics; improve the quality of science education for students and teachers; and enhance public understanding, appreciation, and support

"Only a few of the 'best and brightest' of American youngsters are choosing scientific careers."

of science, science education, and science policy. One of our major means for achieving these goals is ORNL's Ecological and Physical Sciences Study Center. Started in 1984, the Study Center is largely the "brainchild" of Pat Parr, manager of the Environmental Sciences Division's (ESD's) National Environmental Research Park. During the first year of operation, study units in the life sciences were presented to 125 students and teachers at the Study Center. That number has grown tremendously; by 1988 nearly 20,000 local students and teachers had visited the Study Center. In fact, the demand for its use has outstripped our resources, and the number on the waiting list for participation in Study Center activities now nearly equals the number who actually take part!

Under the direction of Kathy Blair, who coordinates all Study Center activities, the popular study units and "hands on" science experiences are provided by ORNL staff members and highly qualified, experienced science teachers. These units have been developed to enhance, rather than replace, traditional classroom science. At the Center, students have the opportunity to experience science as a dynamic and exciting process, rather than a dull collection of textbook facts.

National Programs

Some of our precollege activities are part of outstanding national educational programs. The High School Science Honors Program, sponsored by DOE's Office of Energy Research, brings to participating national laboratories some of the best high school students selected from across the nation and from several foreign countries. In 1988, each state and participating country sent one student to ORNL for a two-week research experience in environmental science. The students joined staff scientists in projects investigating environmental issues such as the global carbon dioxide/greenhouse effect, hazardous waste management, and air pollution problems. The students selected for this program are housed at Maryville College and share the use of its recreational facilities. During their stay at the laboratory, students are involved in research activities, lectures, seminars, role playing, and social activities. The marvelous success of this program is due, in large measure, to the commitment and leadership of ESD staff members. More than 100 contributed their time and effort to help out in 1988.

We also participate in Project SEED, a national program sponsored by the American Chemical Society (ACS), with the primary goal of encouraging financially disadvantaged students to consider careers in science and mathematics. Students selected for the SEED program receive a $1000 stipend, partially supplied by the ACS. Each student is matched with an ORNL mentor for a 10-week summer research experience. As part of the experience, students participate in career education activities and submit a written report on their research. This program is growing—from only one SEED student at ORNL in 1988 to nine students who want to participate in 1989 if funding is available.

Local Programs

Several locally developed programs have been highly successful in enriching science education at area schools. The Special Honors Program for High School Students provides research experiences for talented local youngsters throughout the academic year. Science mentors at ORNL interact with the students by helping them develop a research plan and establish an appropriate experimental approach to the problem. Albert Wong of Oak Ridge High School was ORNL's first participant in this program. His research on artificial intelligence, done with mentor George Geist of the Engineering Physics and Mathematics Division, not only placed first in the 1987 Southern Appalachian Science and Engineering Fair but also placed third in Westinghouse Corporation's prestigious National Science Talent Search. Through the Special Honors Program, students have done research ranging from calculating the diffusive loss in a rippled tokamak to determining the effects of snail grazing on a variety of aquatic plants. Hannah Pigman, a 1989 graduate of Clinton High School, was one of this year's outstanding students. Her progress in understanding electronic circuit systems during her collaboration with mentor Gary Alley of the Instrumentation and Controls Division led to an employment offer to continue the research at ORNL throughout the summer of 1989.

A new local program with great potential is called Partnerships at the Laboratory in Science (PALS). Begun as a partnership arrangement between the Oak Ridge Schools and ORNL, this program provides a formal means for teachers to communicate their needs and for us to find ORNL resources that will match those needs. Through PALS, we have set up a corps of ORNL staff members willing to act as speakers and demonstrators in school classrooms and are now implementing an equipment loan program to supplement the often meager school science equipment budgets. PALS mentors at ORNL are also helping students carry out an ongoing research effort to characterize the cedar barrens area located near Jefferson Junior High School. In addition, we are becoming more involved in the various school "invention" programs, such as Invent America and Invention Convention.

As the benefits of the PALS program become more widely known, we are receiving requests from other school systems who would like to be involved. Because we believe these interactions are important opportunities for ORNL to influence the quality of science education in our area, we are responding to these requests.

"At the Center, students have the opportunity to experience science as a dynamic and exciting process, rather than a dull collection of textbook facts."

Special Programs

Many of our special projects have immediate and visible results. For example, many local school groups attend ORNL events held annually to recognize National Chemistry Week and National Science and Technology Week. Along with the University of Tennessee, we are involved in carrying out the annual Junior Science and Humanities Symposium and the Southern Appalachian Science and Engineering Fair.

A Science Bowl for area high school students was started this year, in cooperation with staff members of Pellissippi State Technical Community College, and we plan to make this an annual event.

Special Study Center programs for minorities and for females were held at ORNL during the 1989 spring break of area schools. Their success has encouraged us to expand these activities in the future.

During the summer of 1989, we are initiating a new program to provide two one-week summer science experiences for local youngsters (3rd through 7th grades). More are being planned for the summer of 1990.

Retired ORNL physicist Bob Kernohan lends a hand at Balloon Day during National Science and Technology Week.

Staff Involvement

As already mentioned, the success of all our precollege activities depends primarily on the willingness and dedication of participating staff members. Because of the outstanding generosity of the ORNL staff, I have been able to respond to almost every request from area schools. Despite their busy lives, the scientists here seem always willing to monitor a research project, speak to a class, judge a science fair, or help a teacher develop or improve science curriculum. Though there is no monetary reward for this involvement with students and educators, our scientists seem to enjoy passing on some of their enthusiasm and enjoyment of science.

There are many sound national reasons to encourage youngsters to study science—to forge a strong economy, to maintain our technological leadership, to build a strong defense. But if you ask a scientist why he or she chose a career in science, the answer is almost always the same—because it's fun! We can provide no greater encouragement to the future of science in America than by sharing the fun and excitement of science with the next generation.

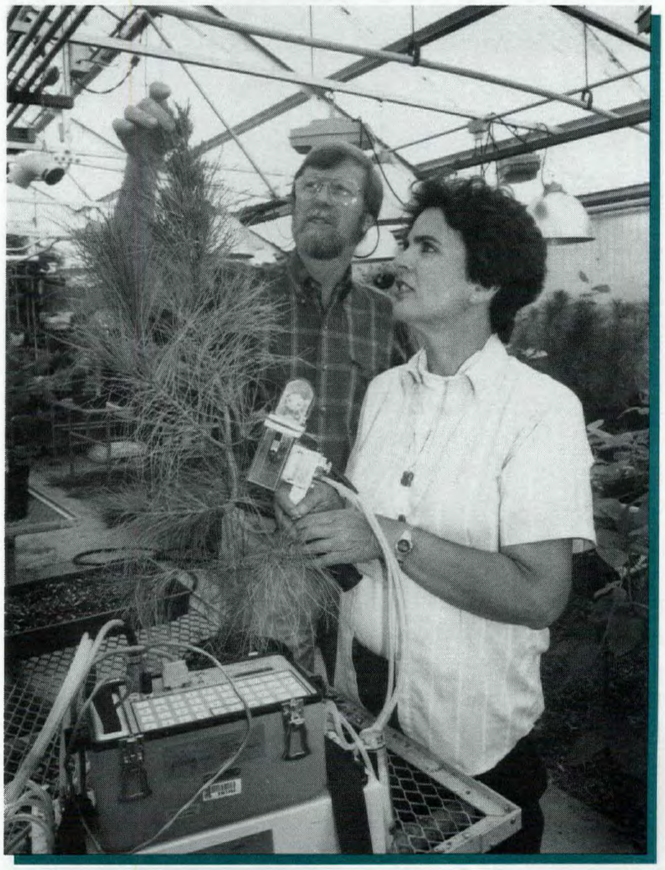
Goals for the Future

Because of the outstanding response and growing interest in our Study Center activities, we hope to be able to expand them. Plans are being made to develop and present additional study units and more general interest programs aimed at increasing public understanding and support for science and science education. More units designed to provide family-oriented science experiences are especially needed, because there is clear evidence that working through the family network improves the achievement levels of youngsters and the attitudes of their parents toward science. We also see a need for units designed especially for handicapped individuals and activities designed to attract and interest more females and minorities in science careers. Because mathematics is a primary tool of many sciences, we plan to develop additional study units in that area also.

We want to affect the teaching of science throughout East Tennessee in a positive way. Some efforts are already under way in this area; we interact with teachers by introducing them to the world of research through summer workshops, by loans of special equipment for classroom use, by consulting with teachers and administrators on curriculum development, and by offering seminars and "hands on" experiences for teacher groups at the Study Center.

The "Jane Whitaker story" illustrates the widespread positive effects that our teacher development activities can have. Jane is an outstanding science teacher and chairman of the science department at nearby Lenoir City High School. ORNL staff members helped her develop curriculum materials on superconductivity—certainly a topic at the forefront of material science studies today. Jane did not stop with using these materials in her own classroom. She has also conducted both local and national teacher workshops on superconductivity, and the materials we helped her develop were distributed nationally by DOE during National Science and Technology Week. We believe the "ripple effects" from teachers like Jane who gain from our programs can significantly improve science education in this area, and even nationally.

ORNL also participates in the Teacher Research Associate Program, a DOE-funded summer research opportunity that provides a stipend and housing allowance for teachers selected on a national level. Other teachers take part in a similar program called STRIVE (Science Teachers Research for Vital Involvement), which is a cooperative venture of Oak Ridge Associated Universities and ORNL.



ESD staff member Nelson Edwards shows teacher Diane Fellows of Clinton how to measure the effects of acid rain and ozone exposures on the needle pigments of loblolly pine.

In an effort to expand our teacher training activities, we have helped develop a multilaboratory proposal to the National Science Foundation requesting support for a program to utilize the facilities of the national laboratories for teacher development. In this program, every participating laboratory would develop training activities in an area of particular strength. For example, ORNL would offer training in the materials sciences to kindergarten through sixth grade teachers because of our excellent facilities and outstanding expertise in this area.

Through all of these efforts, we hope to encourage teachers to develop and maintain links with technical staff members at ORNL, establishing an area-wide, or even nationwide, science education support network, connecting the needs of the schools with the resources of ORNL and the other national laboratories. ornl

Linda Cain, who came to ORNL in 1986, is the Precollege and Special Projects Administrator for the University and Educational Programs Office. She holds an M.S. degree in education from Florida Atlantic University, as well as an M.S. degree in chemistry and an Ed.D. degree in science education from the University of Tennessee. A former teacher at Webb School of Knoxville, she has received numerous teaching awards. She was general chairman of the 1988 International Science and Engineering Fair held in Knoxville, and is current president of the Science Association of Tennessee.

Ecology: Organisms and the Biosphere

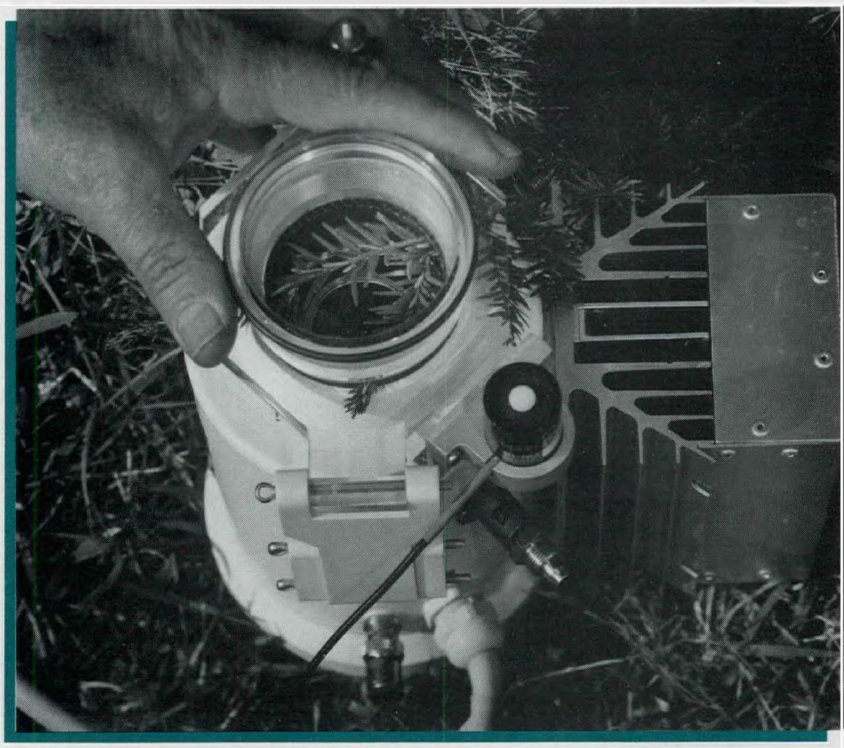
By Carl Gehrs and Robert I. Van Hook

Ecology! The term has its origin in the Greek word "oikos" meaning house. Initially ecology referred to the relationship of an organism to its environment. This relatively new science, first recognized in the early 1900s, has grown and matured. Currently, ecology relates organisms and populations to their environment in terms of process and function, referred to as ecosystem ecology.

ORNL's Environmental Sciences Division has led the development of ecosystem theory and the study of large-scale ecosystems such as the Walker Branch Watershed. Focusing initially on forests in the eastern United States, the theory has expanded to include multiple types of terrestrial and aquatic ecosystems.

Today many ecological studies focus on the larger relationships of ecosystems to landscapes, regions, and the global environment. Full understanding of these relationships necessitates investigations into physiological, biochemical, and molecular origins of the responses of living organisms to, for example, acidified streams and rising levels of atmospheric carbon dioxide.

Within the last decade, ecologists have recognized that both genetic makeup and biochemistry are important ecological factors. Principles that determine the broader relationships of an organism to the population, community, and ecosystem are governed by the molecular responses within the cell. Thus, ecology is beginning to embrace studies of phenomena on a subcellular as well as a global scale. ornl



ESD researchers use sophisticated photosynthesis-measuring devices to study the impacts of atmospheric pollutants on plants.

Carl Gehrs is head of the Environmental Toxicology Section in the Environmental Sciences Division. He received a Ph.D. degree in zoology from the University of Oklahoma and joined ORNL in 1972. He has served as co-chairman of the 8th Life Sciences Symposium: International Conference on Bioindicators. He is advisor to the Minister of Interior, Federal Republic of Germany, on biomarkers and long-term biological monitoring.

Robert I. Van Hook is ESD associate director, manager of the Renewables Program, and leader of ORNL's Global Environmental Studies activities. He came to ESD in 1970, the same year he received his Ph.D. degree in entomology from Clemson University.

Ecology studies phenomena ranging from the subcellular to the global.

ESD staff members examine a map of Walker Branch Watershed streams and forest plots, produced by a large electrostatic plotter of the new Geographic Information System.

This weir is used to monitor stream discharges on Walker Branch Watershed. The flux of elements leaving this watershed in stream flow is determined at regular intervals.

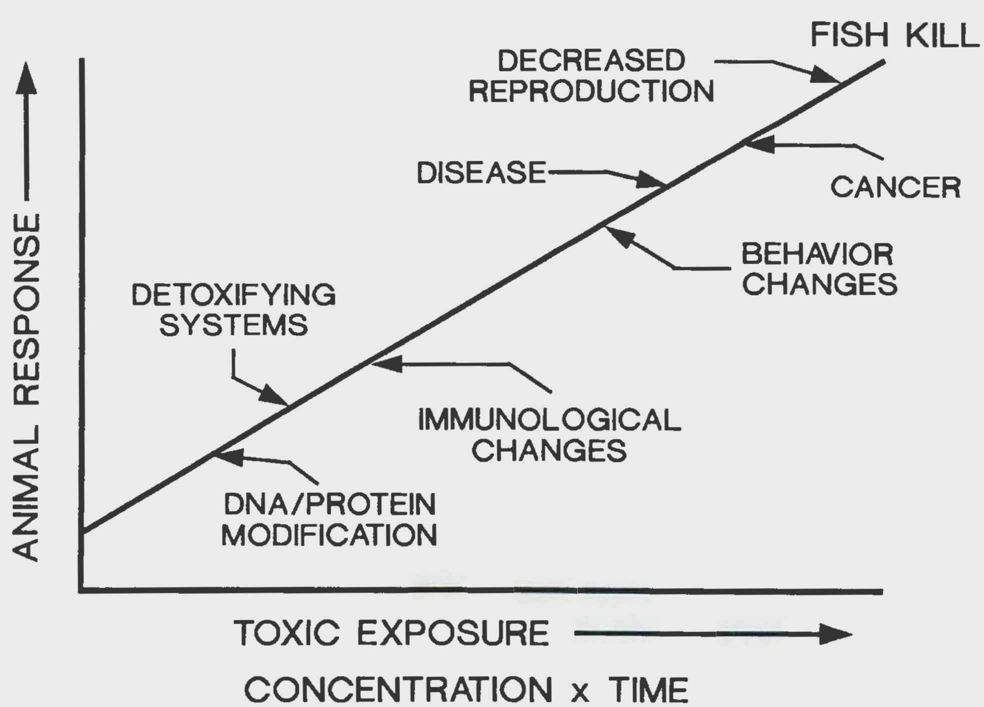
Biomarkers for Pollutants

By John F. McCarthy, S. Marshall Adams, Lee R. Shugart, and Braulio D. Jimenez

Twenty-five years ago, Rachel Carson's *Silent Spring* galvanized public concern about toxic chemicals (e.g., DDT and other insecticides) in the environment. In the interim, landmark environmental legislation has made huge strides in identifying and regulating the release of contaminants. Yet, it is still surprisingly difficult to decide whether measurable levels of contaminants in soil, water, or air pose a long-term threat to an ecosystem or to humans. At a typical waste disposal site or river receiving industrial effluents, detailed chemical analyses of soil, air, water, plants, and fish can yield a laundry list of potentially toxic compounds, most of them at concentrations below clearly toxic "action levels." The total toxic effect depends partly on each chemical's location and ability to enter the organism(s) of concern; for example, a part-per-trillion level of dioxin in water may be more harmful to a fish than a part-per-million level in sediment. Furthermore, the toxic effect of a "brew" of chemicals could not be predicted, even if we understood the action of each component; mixtures may have effects greater than or less than the sum of the toxicities of the chemicals (synergism or antagonism).

Chemical analyses at these low concentrations are very expensive and provide data that not only are difficult to relate to a biological effect but may not even accurately reflect the status of chemical concentrations over time and space. A chemical sampling is a "snapshot" that does not take into account changes that can occur over time or as a result of storm events, shifts in wind direction and

BIOMARKERS: THE CONCEPT



Animal response to toxicant exposure is a function of the concentration of chemical in the environment and the length of exposure. The biomarker approach targets early responses (lower left) that are useful in predicting long-term, irreversible, adverse effects (upper right).

ORNL researchers are studying animals for early biological warning signs of toxic exposure.

velocity, or intermittent releases from industrial plants. Furthermore, contamination is often geographically patchy; a quiet pool in a stream may accumulate highly contaminated silt, whereas the gravel bottom a few feet away may have only trace levels.

Animals as Sentinels

Faced with these complications and uncertainties, we have used animals for evaluating the ecological and health significance of contamination. Our approach is based on the simple premise that the animals in the environment "know" if they are being poisoned and can "tell" us the nature and extent of the poisoning if we look for the right signals, or "biological markers" (biomarkers, for short). A fish kill, the disappearance of key populations in an ecosystem, and cancer in animals are irreversible adverse effects that express themselves after a long period of exposure (see drawing). We are developing methods for looking at early warning signs (expressed as biological responses) that indicate toxic exposure to the animal.

The use of animal responses as toxicity indicators overcomes many of the limitations that plague our chemical analyses of the environment. The animals integrate exposure over time and across a spatial range, and the biological markers represent the sum of toxic exposures, accounting for the complexities of differential uptake from soil, water, or food and the toxicological interactions among complex mixtures of contaminants.

Lee Shugart and Kitty Gustin use a high-performance liquid chromatograph to detect and quantify the amount of benzo[*a*]pyrene chemically attached to the DNA of animals from contaminated environments.

Braulio Jimenez (right) and John McCarthy review enzyme analyses of blood samples from fish in Oak Ridge streams. The centrifugal analyzer (front), developed by ORNL's Chemical Technology Division, automatically loads and analyzes 30 blood samples simultaneously.

Our biomonitoring program for streams in and around Oak Ridge provided the impetus for developing and applying biomarkers in environmental species. Although the program has funds to measure biomarkers in fish from the streams, additional support from the ORNL Director's R&D Fund permitted us to develop new biomarkers, build collaborations with other ORNL divisions and outside institutions, and conduct critical laboratory experiments to demonstrate relationships between contaminant exposure and the responses of specific biomarkers. Most of our research has focused on fish, but we have tested our methods on tissue samples from animals ranging from beluga whales to herring gulls. Several classes of biomarkers are currently being evaluated in field and laboratory research.

DNA Damage

Chemical interaction and modification of deoxyribonucleic acid (DNA) are believed to be the key initiating events in mutagenesis and carcinogenesis. Lee Shugart, of ORNL's Environmental Sciences Division (ESD), adapted techniques he developed while at the Biology Division to quantify DNA adducts [carcinogens, such as benzo[*a*]pyrene (B*a*P), that have chemically attached themselves to DNA] in a wide variety of environmental species. Using this technique, he recently analyzed DNA from the brains of a declining population of beluga whales in the polluted St. Lawrence River of Canada. He found DNA modifications from B*a*P at levels as high as those observed in mice and fish experimentally exposed to a carcinogenic dose of the chemical. In contrast, belugas from a pristine area in Canada's Northwest Territories had no detectable adducts (see sidebar).

Though this technique is powerful for demonstrating exposure to specific agents, there are other ways that DNA can be damaged by genotoxic agents. Two biomarkers have been developed and evaluated to provide a general measure of the integrity of the DNA: one measures the number of breaks in the DNA polymer, and the other measures the level of 5-methyldeoxycytidine (5-mdCyd), a minor nucleotide in DNA that has a critical role in controlling the expression of the specific genes. Our experiments demonstrated that strand breaks in DNA increased in fish exposed to B*a*P and that 5-mdCyd levels declined in fish that were exposed to either B*a*P or cadmium.

Both measures of DNA integrity were affected in animals from a variety of contaminated areas, ranging from fish and turtles in streams receiving effluents from the Oak Ridge Y-12 Plant and ORNL (although improvements in Y-12 waste treatment appear to be reducing these effects on the DNA) to kangaroo rats and coyotes living near an oil drilling operation in the desert of southern California. We are also analyzing samples from cormorants and herring gulls from heavily industrialized areas around Green Bay, Wisconsin.

Induced Detoxification

Many contaminants stimulate the synthesis of protective detoxification systems. The higher level of induced detoxification found in field-collected vs reference-site animals is a biomarker indicating

Marshall Adams measures blood gases in fish experimentally stressed by low-pH water to determine the chronic effects of acid rain.

a molecular response to toxicant exposure.

Braulio Jimenez of ESD has shown that fish in contaminated streams have significantly higher levels of several components of the mixed function oxidase (MFO) system than fish from reference streams. MFO is a polyfunctional enzyme complex found in the liver that is induced by a variety of organic contaminants. Jimenez's work has also illustrated the importance of understanding the influence of environmental variables in the application and interpretation of biomarkers. For example, the MFO system is more responsive to contaminant exposure in fish acclimated to warmer temperatures; consequently, care must be taken in comparing results from fish collected in different seasons or from waters of differing temperatures. Cellular damage to the liver resulting from either the presence of cytotoxic agents or heavy infestations of parasites can not only alter the capacity of the MFO system to be induced but also cause the release of hepatic enzymes into the bloodstream. None of these complications prevent the use of the system as a biomarker, but these interactions must be considered to properly interpret the responses.

Another detoxification system exists in animals exposed to toxic metals. Exposed organisms will synthesize higher-than-normal levels of the low-molecular-weight, metal-binding proteins (metallothioneins), which prevent metals from entering cells and exerting toxic effects. Bruce Jacobson of the Biology Division has been collaborating with us to modify and improve a technique he developed for studying metal metabolism in the fruit fly, *Drosophila*, and measuring levels of metallothioneins in fish. Using this rapid procedure, we have demonstrated that fish exposed to metals in the laboratory and fish from metal-contaminated streams have significantly higher levels of metallothionein than fish from pristine streams.

Immune System and Population Effects

Guy Griffin, of the Health and Safety Research Division, is collaborating with us to develop and evaluate biomarkers of immune system function. He is developing an assay to measure interferon levels in fish and to quantify the effects of contaminants on the protective increases in interferon levels that occur when fish are challenged with a virus.

One of the key parameters in predicting the population-level effects from toxicants is reproductive competence (i.e., the number of healthy offspring produced). Mark Greeley, a fish-reproduction physiologist in ESD, is developing crucial links between toxicant exposure and population-level effects in an ecosystem. Reproductive success is related not only to the direct effects of toxicants on critical molecular, biochemical, and endocrinological functions, but also to indirect effects (e.g., changes in the food chain of the organism can affect the accumulation of energy reserves necessary for reproduction).

Marshall Adams of ESD has been evaluating a number of biochemical, physiological, and tissue- and organ-level parameters such as blood chemistry, histopathology, lipid storage, RNA/DNA ratios, and growth, as biological indicators of population-level effects (see photograph on p. 85). He has been comparing data from field surveys of fish in streams around Oak Ridge with laboratory data to examine the effects of contaminants on respiratory physiology and bioenergetics of critical life processes. Adams also leads field expeditions to examine fish from areas contaminated with a range of chemicals. He and Jimenez have sampled portions of the Elizabeth River near Norfolk, Virginia, which is polluted by carcinogens from an old creosote factory, as well as areas of the Pigeon River, which receives effluents from a pulp and paper mill in North Carolina. An entire suite of biomarkers, from the biochemical to the population level, are being measured for these fish.

Advantages of Biomarkers

The various classes of biomarkers can provide information on environmental contamination in several ways:

- Animals can act as *sentinels* of bioavailable contaminants, because they integrate exposure spatially, temporally, and cumulatively to provide data that can complement and extend the usefulness of chemical survey data. Signals of exposure, either biomarkers or measured concentrations of persistent contaminants in the animals, can identify "hot spots" of toxic chemicals in the environment. This information can help determine the order in which waste sites should be cleaned up, document the effectiveness of remediation, or just warn of the potential for human exposure.

- Animals can act as *surrogates* for human exposure. Animals collected, for example, around hazardous waste sites or in adjacent areas of human population are helpful in evaluating human health effects. Animals can identify locations of bioavailable contaminants and help define areas of exposure for human populations. Animal surrogates are free from the lifestyle (e.g., cigarette smoking) and workplace factors that complicate and confound epidemiological investigations in humans. Human risk assessments could be improved by extrapolating doses measured in animal surrogates to equivalent levels in humans through the use of metabolic models such as those described by Curtis Travis (see p. 187). Biomarker responses in animal surrogates also provide information on the consequences of chronic exposure to chemicals in the environment that can contribute to understanding the relationship between exposure and disease in humans.
- Biomarkers in animals can be *predictors* of ecological effects. The long latent period between exposure to toxicants and the expression of ecological effects (for example, the loss of key commercial or sports fisheries or decline of an endangered species) has complicated environmental protection and prevented intervention before the damage becomes irreversible. Ultimately, we would like to use biomarker responses as short-term predictors of long-term ecological effects.

In summary, the approach we are developing and applying at ORNL is to use molecular, biochemical, and physiological biomarkers in animals to detect and assess the effects of contaminants in the environment. Our research has addressed specific problems in assessing the effects of pollutants and the effectiveness of improved waste treatment on the health of aquatic organisms in Oak Ridge Reservation streams. We will soon be tackling similar questions of chemical exposure and effects in mammals in a desert environment. Biomarker research, an applied problem, also contributes to our basic understanding of the actions and interactions of toxicants in critical target tissues, whether they be in fish, herring gulls, or beluga whales. ornl

John McCarthy is leader of the Biological Chemistry Group in the Environmental Sciences Division. He received a Ph.D. degree in biological oceanography from the University of Rhode Island and did postdoctoral work at ORNL's Biology Division before coming to ESD in 1980.

Marshall Adams has been an ESD research staff member since 1974. He has a Ph.D. degree in marine sciences from the University of North Carolina.

Lee Shugart is an ESD research staff member and an adjunct faculty member of the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences. He received his Ph.D. degree in microbiology from the University of Tennessee and did postdoctoral work in ORNL's Biology Division.

Braulio Jimenez is an ESD research associate and an ad honorem professor at the University of Puerto Rico. He received his Ph.D. degree in marine sciences from the University of Puerto Rico and then worked as director of the Office of Environmental Research for the Environmental Quality Board of the Puerto Rican government.

ORNL Study Interests Canadians

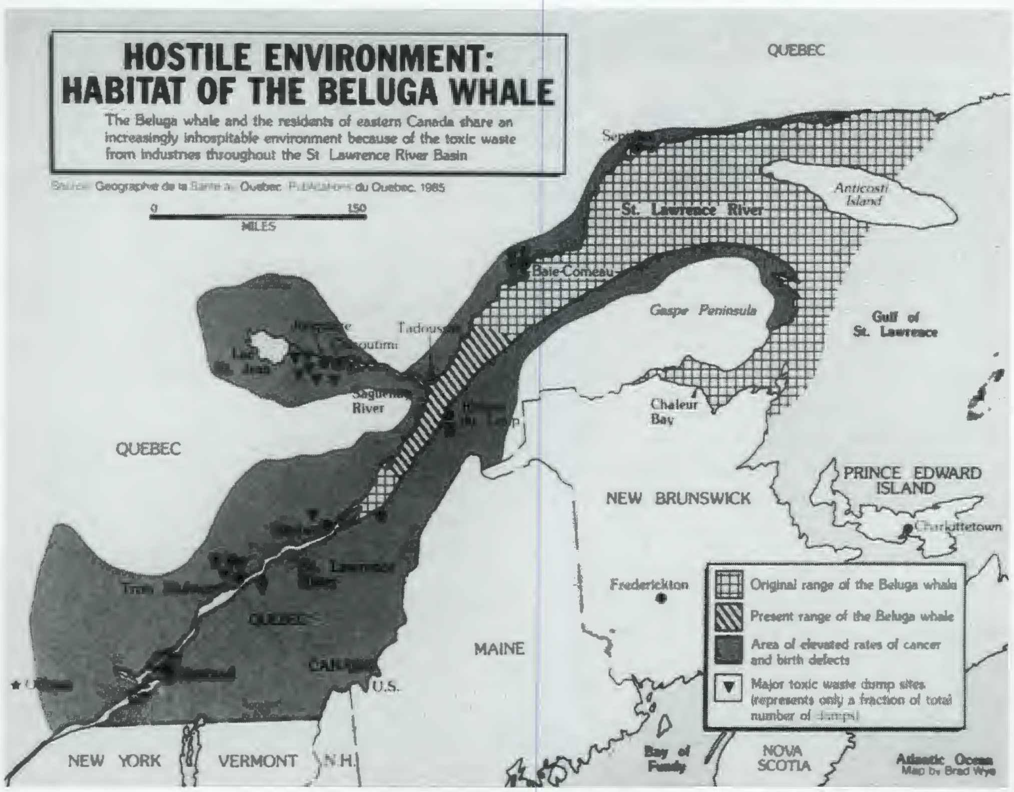
Lee Shugart's studies at ORNL documenting the exposure of an endangered Canadian beluga whale population to a chemical pollutant have attracted the attention of several Canadian groups. Officials in Environment Canada (the Canadian equivalent of the U.S. Environmental Protection Agency) have called him about his results. Representatives from the Aluminum Company of Canada (ALCAN), which has been blamed for polluting the area where the whale population is declining, have visited Shugart's laboratory. In the fall of 1988, he presented his findings to the International Forum for the Future of the Beluga Whale, held in Tadoussac, Canada.

In addition, a Canadian film crew for Greenpeace, the international environmental activist organization, has made part of a film at ORNL that will be shown on lake tour boats and offered to television networks in Canada. And Shugart's work has been widely reported in the popular press, including the Montreal *Gazette*, *Greenpeace* magazine, the *New York Times*, and the science page of the Knoxville *News-Sentinel*.

In 1987, Canadian scientists asked Shugart to test beluga whale tissue samples, using a highly sensitive technique he had devised for analyzing tissue of chemically exposed animals. The scientists were concerned about a population of about 300 beluga whales in the St. Lawrence and Saguenay rivers that continued to decline even after hunting was prohibited. Autopsies indicated that the whales were dying of various diseases, possibly the result of weakened immune systems. Because 30 different toxicants had been found in the blubber of these whales and in river eels (the whales' main food), it was suspected that regional pollution might be jeopardizing whale health.

Canadian scientists had analyzed the air, sediments, and water in the region and found insecticides, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons, including

A Canadian autopsy program found high levels of 24 different contaminants in the flesh of St. Lawrence River beluga whales. (Photo credit Richard Bourassa)



The endangered beluga whale continues to decline in its polluted environment. (Source: *Greenpeace* magazine)

benzo[*a*]pyrene (B*a*P), a potentially cancer-causing compound present in cigarette smoke, automobile exhaust, and industrial emissions. They speculated that B*a*P was a contributing factor to the whales' demise. One source of this pollutant is the regional aluminum smelter operated by ALCAN. The company's stack emissions have already been blamed for a higher-than-expected incidence of bladder tumors in ALCAN workers and of cancers and birth defects in surrounding communities.

To determine whether beluga whales in the St. Lawrence River had been exposed to B*a*P, Shugart obtained permits to import brain tissue from three dead whales. Using a fluorescence technique he developed in 1983 at the Biology Division, Shugart sought evidence that genetic material (DNA) in the brain tissue had been modified chemically by a metabolite of B*a*P, the diolepoxide formed as the whale's natural response to detoxify B*a*P. He measured the amount of the metabolite that had been chemically bound to DNA. A metabolite bound in this way is called an adduct and is believed to signal the initial events of carcinogenesis. In his technique, the metabolite attached to the whale DNA is chemically removed and exposed to ultraviolet light, causing it to fluoresce. Because his fluorescence technique is sensitive enough to detect one DNA modification in ten million nucleotides (DNA building blocks), Shugart could quantify the DNA adducts and thus estimate the whales' level of exposure to B*a*P.

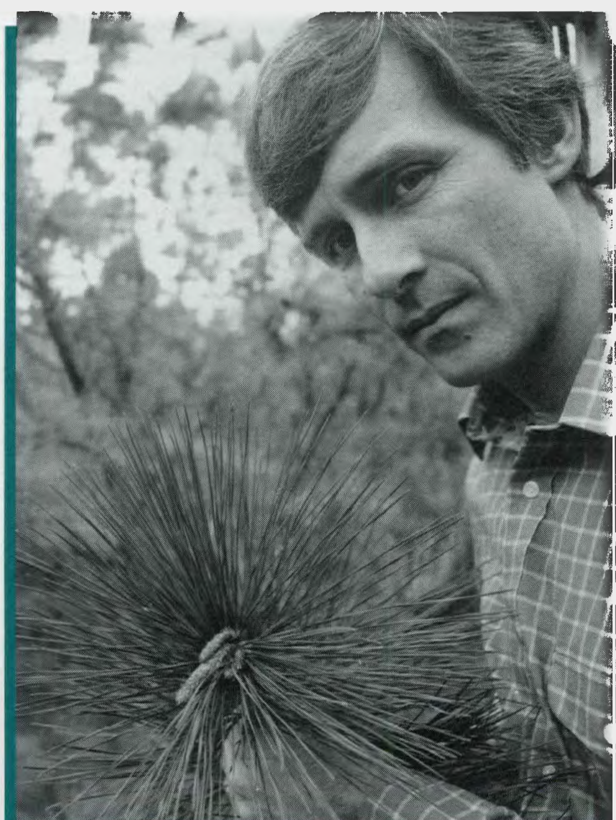
Shugart showed not only that the three beluga whales had been exposed to B*a*P but also that their level of DNA modification was similar to that observed in experimental animals that had received a B*a*P dose high enough to produce tumors. Similar analyses on beluga whales from an uncontaminated environment in the Northwest Territories of Canada showed no evidence of exposure to B*a*P.

Shugart developed the analytical technique used for this study with support from the ORNL Director's R&D Fund.

Atmosphere–Canopy Exchange in Forests

By George Taylor, Steve Lindberg, Paul Hanson, and Chuck Garten

In both the physical and life sciences, the exchange processes that occur at transitions between phases (e.g., gas-liquid, liquid-solid) are of fundamental importance. In terrestrial landscapes, one of the most pronounced transitions is that between the atmosphere (gas phase) and biosphere (liquid and solid phases). Forest trees create a highly visible and important boundary layer in which the inertia of the turbulent free atmosphere is dissipated, and molecular-level interactions begin to control exchange processes. Research in the Environmental Sciences Division (ESD) at ORNL is addressing multiple aspects of exchange at this atmosphere-canopy interface, including the deposition to trees of atmospheric pollutants such as sulfates and the emission by trees of trace amounts of sulfur-containing gases.



Steve Lindberg, one of the authors, was recently elected chairman of the U.S. National Atmospheric Deposition Program.

Studying atmosphere–forest canopy interactions is an interdisciplinary effort involving atmospheric chemistry and physics, biogeochemistry, meteorology, toxicology, and plant physiology. An understanding of exchange processes at the atmosphere-canopy interface is important in assessing

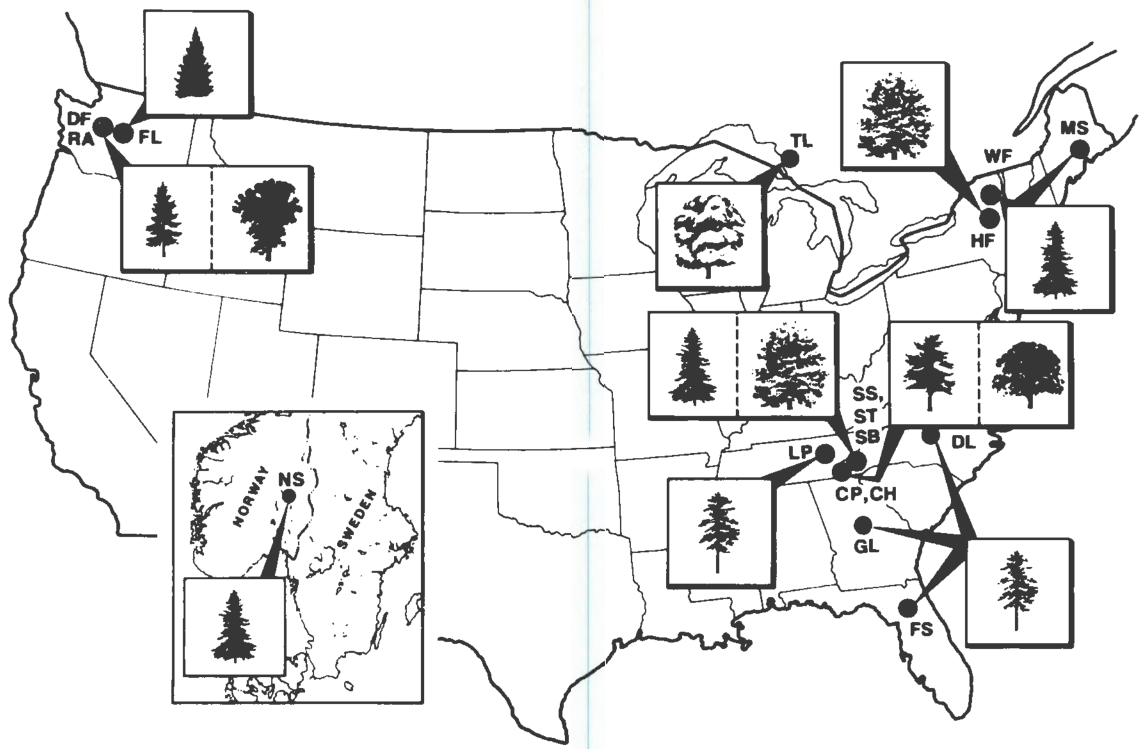
- the biogeochemistry of terrestrial landscapes;
- the role of the biosphere as a source of and sink for trace gases, which influence the chemical and physical properties of the atmosphere and, thus, affect global climate; and
- the influence of atmospheric pollutants on the physiology and growth of forest trees and agricultural crops.

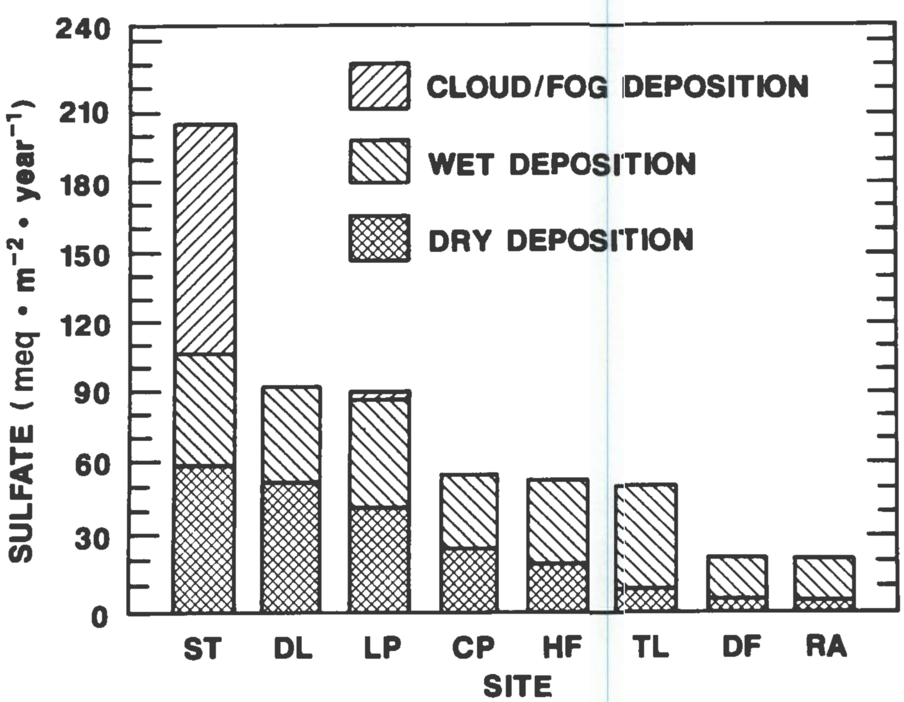
Atmospheric Deposition to Forests

The effects of atmosphere-canopy exchange processes on the biogeochemistry of terrestrial landscapes have received increasing attention in the last decade, with the recognition that prolonged atmospheric inputs of sulfur, nitrogen, and hydrogen might influence the nutrient cycling and productivity of forests. One of the major issues to be resolved is the spatial distribution of these atmospheric inputs, reflecting the combination of marked differences in pollutant concentrations among regions and the differential scavenging efficiency of forest canopies for atmospheric gases and particles.

To more fully understand this issue, the Integrated Forest Study was conceived and is being managed by ORNL scientists Steve Lindberg and Dale Johnson. The objective of this project funded by the Electric Power Research Institute is to intensively characterize the atmospheric deposition and nutrient cycling at 13 forest sites in the United States, Canada, and Europe (see figure on next page). Through a comparison across sites, we can then investigate the processes

ESD researchers advance our understanding of pollutant gas effects on forests.





The EPRI Integrated Forest Study's goal is to characterize the atmospheric deposition and nutrient cycling at 13 forest sites in the United States, Canada, and Norway. The sites named on both the graph and map are Smokies Tower (ST), Duke Loblolly (DL), Loblolly Pine (LP), Coweeta Pine (CP), Huntington Forest (HF), Turkey Lake (TL), Douglas Fir (DF), and Red Alder (RA). Other sites on the map are Findley Lake (FL), Smokies Spruce (SS), Smokies Beech (SB), Coweeta Hardwood (CH), Georgia Loblolly (GL), Florida Slash Pine (FS), Whiteface (WF), Maine Spruce (MS), and Norway Spruce (NS).

governing deposition and subsequent element cycling within the forest.

The magnitude of variation among sites in atmospheric deposition of chemical species is clearly demonstrated by that of sulfate, in which inputs differ by more than an order of magnitude from a minimum in a red alder stand in Washington State (RA) to a maximum in a spruce forest (ST) in the Great Smoky Mountains National Park (see graph). This variability in sulfate deposition is a consequence of the site-specific parameters of local and regional air quality and the physical forms of deposition as cloud-fog water interception, wet precipitation, and dry deposition of gases and particles.

Of particular interest to scientists studying the role of atmospheric deposition as a stress in terrestrial ecosystems is the effect on productivity and nutrient cycling in high-elevation forests in North America and Western Europe, where forest decline is most evident. For comparable concentrations of atmospheric pollutants, the rate of deposition of airborne chemicals to plant canopies at higher elevations tends to be higher than that to forests at low elevations. This difference is a consequence of higher precipitation rates (induced by mountains), higher wind speeds, prolonged cloud immersion, and greater foliage surface areas.

Steve Lindberg, Doug Schaefer, and Jim Owens in ESD's Geosciences Section, in association with National Park Service staff, have established some deposition measurement sites in the southern Appalachian Mountains, where meteorological and chemical data are being collected to compare rates of wet and dry deposition to conifer forests. The high-elevation (1800-m) site is in a spruce forest near Clingmans Dome in the Great Smoky Mountains National Park (ST), and the low-elevation (300-m) site is in a loblolly pine forest on the Oak Ridge Reservation (LP).

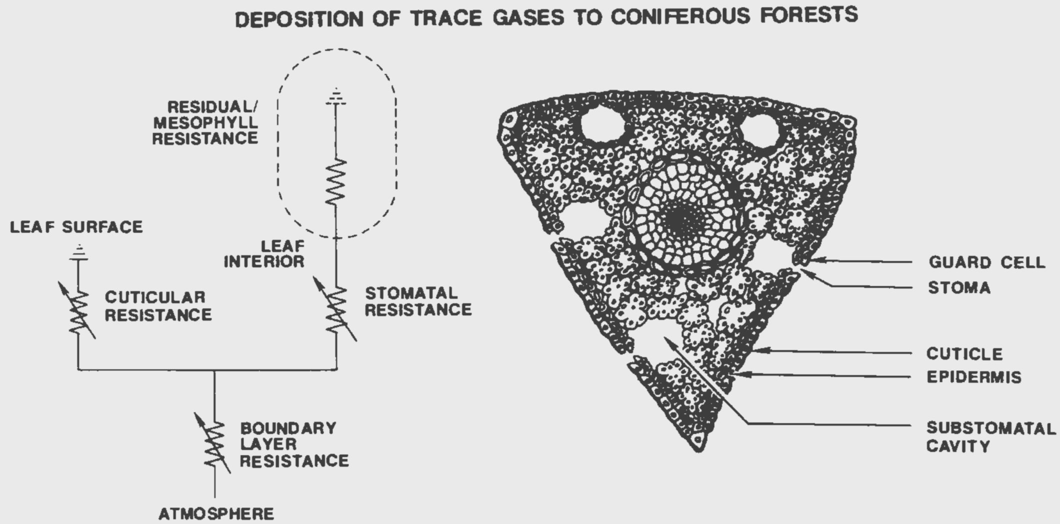
Meteorological measurements confirm the expected differences in atmospheric exposure conditions between the Great Smoky Mountains spruce and the Oak Ridge pine sites. Annual precipitation at the spruce site is nearly twice that at the pine site, whereas wind speed during cloud immersion at the spruce site exceeds that at the pine site by a factor of 50. Estimates indicate that the spruce stand has 2 to 4 times as much foliage area exposed to atmospheric deposition as the pine stand.

Atmospheric concentrations of airborne particles and nitric acid (HNO_3) vapor are comparable between sites, whereas sulfur dioxide (SO_2) levels are 50% lower at the mountain site. Ion concentrations in rain are about a factor of 2 lower in the mountains. The most striking differences are found in fog and cloud chemistry. Fog in the valley is generally a nonacidic solution dominated by salts of calcium and ammonium sulfate, whereas cloud water impinging on the mountains is a highly acidic solution dominated by HNO_3 and sulfuric acid.

Data on the total annual atmospheric deposition to these sites confirm the hypothesis that atmospheric fluxes increase from the valley pine to the mountain spruce site, despite the fact that the mountain site is farther away from major emission sources in the Tennessee Valley. Total wet plus dry deposition at the mountain (ST) site is at least a factor of 2 higher than at the valley (LP) site for all of the major atmospheric chemicals measured. In fact, deposition of the major ions associated with acidic deposition is higher at the Great Smoky Mountains site (mountain site) than at any other site in the Integrated Forest Study (see graph).

Deposition of pollutants to the mountain site occurs because of rainfall (wet deposition) and because the tree foliage scavenges particles, gases, and cloud water from the atmosphere (dry and cloud deposition) more effectively. Until recently, dry and cloud deposition processes have been largely ignored in forest ecosystem studies. These deposition data are now being combined with soil chemistry and nutrient cycling data to determine the effect of deposition on normal biogeochemical processes in forests. Preliminary results suggest that atmospheric deposition increases the leaching of beneficial nutrients from tree foliage and forest soils and promotes conditions leading to forest plant uptake of potentially toxic aluminum.

This 120-ft tower is used for collecting dry sulfur deposits from the air before they reach the forest canopy level.



Trace pollutant gases from the atmosphere are absorbed by the leaves of coniferous trees, where they reach interior leaf tissues through microscopic pores called stomata.

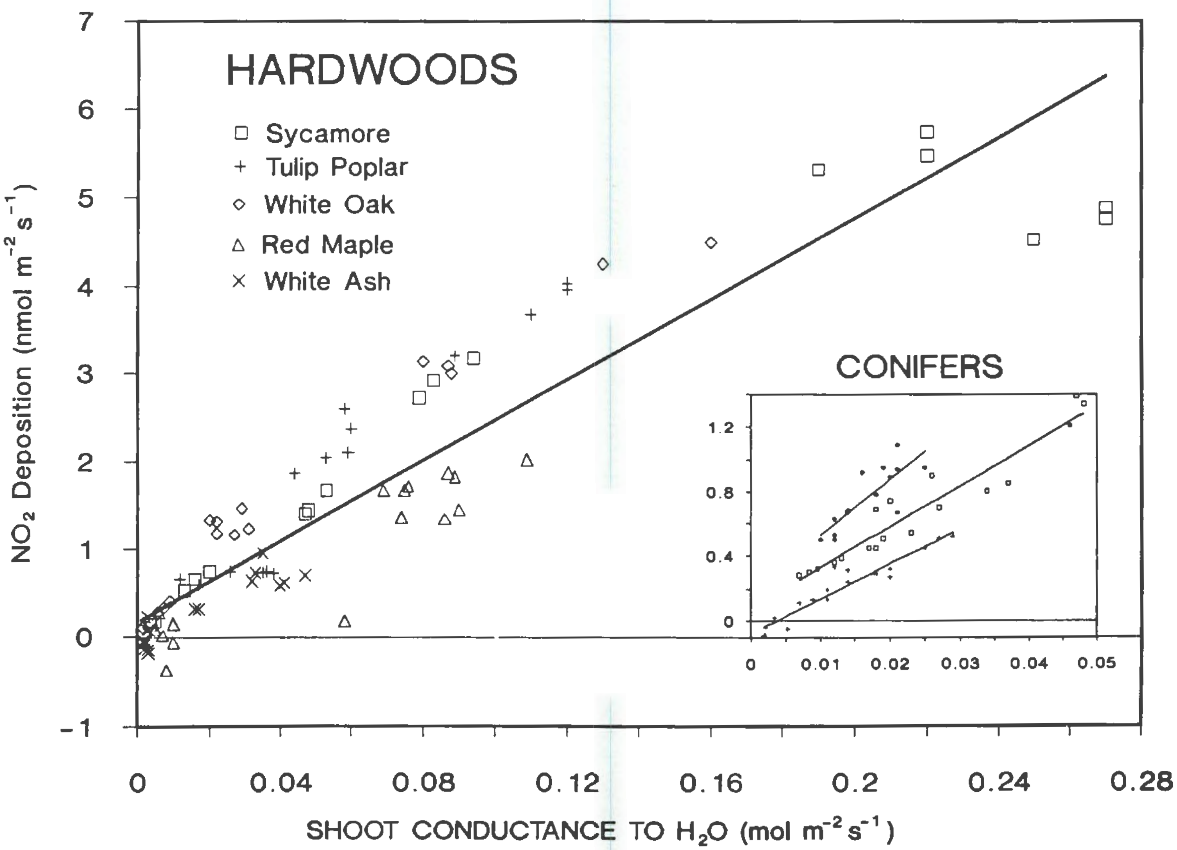
Absorption of Pollutant Gases

Pollutant gases are absorbed by plant canopies because a chemical gradient exists between the atmosphere surrounding individual leaves and the sites of absorption, either on the leaf surface or in the leaf interior (see figure above). Interior tissues of the individual leaves are accessible to pollutant gases through microscopic pores (stomata) that regulate the bidirectional exchange of gases. Stomata serve as highly sensitive servomechanical devices similar to a thermostat.

The unique structural and functional properties of stomata prompted scientists in ESD's Physiological Ecology Group (Paul Hanson, Carla Gunderson, and George Taylor) to propose a new concept of leaf-atmosphere exchange based on the two-layer stagnant film model developed decades ago in chemical separation science. In the model, phase transition occurs at the gas-liquid interface in the leaf interior and the partitioning of molecules across this interface is based on Henry's law, a basic principle of physical chemistry. This research is supported by DOE's Office of Health and Environmental Research.

One of the most significant corollaries of this model in physiological plant ecology is that the leaf functions as a chromatographic column, separating chemical mixtures of gases into individual components as they diffuse into the leaf. Thus, pollutant gases are absorbed at different sites in the leaf interior, depending on their physicochemical properties in both the gas and liquid phases. Water-soluble gases (e.g., SO_2) are extracted immediately as they diffuse into the leaf interior, and less soluble gases (e.g., ozone) permeate the leaf interior more uniformly and deeply. This pattern helps explain two long-standing issues in the physiological ecology of pollutant gases: (1) the tissue-specific toxicity of pollutant gases with respect to leaf functions and (2) patterns of metabolic dysfunction resulting from the interactive effects of pollutant mixtures.

At the leaf level, absorption is controlled by a combination of physiological and environmental factors, each having a highly specific role. To investigate these phenomena as part of EPRI's Integrated Forest Study, we measure absorption of trace gases in our Glass Dome Reactor System, a unique controlled-environment facility. For example, nitrogen dioxide (NO_2) is a common constituent of the troposphere and plays a major role in atmospheric chemistry, nutrient inputs in terrestrial landscapes, and plant physiology. Although atmospheric concentrations are well characterized, the atmosphere-to-vegetation transfer is not. In the Glass Dome Reactor System, we have clearly demonstrated that NO_2 absorption in both



The absorption of NO_2 by tree leaves is controlled by the physiological functioning of the stomata. ORNL researchers can model NO_2 deposition to forest canopies simply by tracking stomatal physiology.

hardwoods and conifers is controlled by the physiological functioning of the stomata—when stomata open, NO_2 absorption increases substantially; when stomata close, the leaf is no longer a sink for the gas. Our data are shown in the graph on this page. Because this pattern is common across species, we can model NO_2 deposition to forest canopies simply by tracking stomatal physiology.

Canopies As Sources of Trace Gases

More than 25 years ago, ESD ecologists pioneered the use of radioisotopes to study biogeochemistry in forest stands. More recently, Chuck Garten of the Ecosystems Studies Section has used a short-lived radioisotope of sulfur (sulfur-35) to learn more about how forest trees adapt to the increasing sulfur deposition associated with acid rain. Small amounts of sulfur-35 are injected into mature deciduous and coniferous trees to trace the behavior of stable sulfur. These experiments have answered one question about the behavior of sulfur in trees and raised another, somewhat unexpected, issue.

Garten's studies of the isotope showed that the leaching of sulfur from inside tree leaves by rainfall is small. Thus, the commonly observed increase of sulfur concentrations in rainfall collected below forest canopies (called throughfall) results primarily from the "wash off" of particle and gaseous sulfur deposited and adsorbed on the leaf surface. In the past, quantification of sulfur deposition to forests was difficult, because the architecture of the forest canopy is so complex and variable among sites. With Garten's findings, ecologists have a new means for investigating and quantifying the deposition of sulfur to forest canopies.

The sulfur-35 experiments have raised new questions about atmosphere-canopy interactions—namely, the extent of gaseous sulfur emissions from forest trees. Laboratory studies have previously shown that leaves emit volatile sulfur compounds in response to environmental sulfur exposure. This process, called biogenic emission, is thought to be a detoxification mechanism. From the sulfur-35 studies, it is clear that sulfur is released from the canopies of deciduous and coniferous trees through volatization (see figure on previous page), and this finding leads to the hypothesis that biogenic emission is an important mechanism by which forest trees physiologically adjust to excess sulfur in their environment. Moreover, it suggests that vegetation canopies may be a significant biogenic source of trace gases such as hydrocarbons (in general) and sulfur-containing gases (in particular).

Characterizing the extent, magnitude, and spatial distribution of this trace-gas production is important in understanding long-term changes in the physical and chemical properties of the atmosphere on a global scale.

Many aspects of atmosphere-canopy interactions remain unresolved. For the immediate future, the most important issues in atmosphere-canopy exchange are its role in global climate change and biogeochemistry and the physiological mechanisms underlying the effects of pollutant gases on forest trees. ornl

The authors are researchers in three sections of ORNL's Environmental Sciences Division—Ecological Toxicology, Ecosystems Studies, and Geosciences.

George Taylor, who has been an ORNL staff member since 1979, earned his Ph.D. degree from Emory University. He was a recipient of the National Academy of Sciences–National Research Council Postdoctoral Fellowship in Plant Physiology and is a member of the Clean Air Science Advisory Committee for the U.S. Environmental Protection Agency. He serves on the editorial board of the *Journal of Environmental Quality*.

Steve Lindberg, who received his Ph.D. degree from Florida State University and came to ORNL in 1974, is a co-author of the book *Atmospheric Sulfur Deposition*. His awards include the Humbolt-Stiftung Foundation Fellowship Award and several Martin Marietta publication and achievement awards. In 1989 Lindberg was elected chairman of the U.S. National Atmospheric Deposition Program.

Chuck Garten, who has been at ORNL since 1976, has an M.S. degree from the University of Georgia.

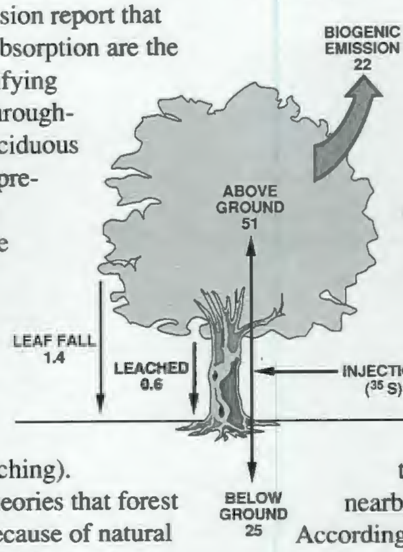
Paul Hanson came to ORNL in 1986 after receiving his Ph.D. degree from the University of Minnesota.

The authors acknowledge the contributions to this article of Carla Gunderson, Doug Schaeffer, and Jim Owens.

Linking Atmospheric Sulfur to Soil Acidification

Recently, ORNL researchers have documented a critical link between atmospheric deposition (including wet precipitation and dry deposition) on tree foliage and acidification of forest soils.

In an article published in the November 10, 1988, issue of the British journal *Nature*, Steve Lindberg and Chuck Garten of ORNL's Environmental Sciences Division report that atmospheric deposition and absorption are the dominant sources of the acidifying sulfur compounds found in throughfall beneath both pine and deciduous trees in the Southeast. Some previous studies have concluded that sulfur in throughfall—the rain washing off previously dry-deposited and surface-adsorbed sulfur compounds from leaves and needles —was strongly influenced by the natural leaching of internal tree sulfur (foliar leaching). The finding casts doubt on theories that forest soils are enriched in sulfur because of natural discharges by trees of sulfates taken up from the soil (recycling). It had been proposed that sulfates in leaves and needles on trees or the forest floor are leached out by precipitation (foliar leaching).



Using a sulfur-35 tracer, the ORNL researchers determined that more than 95% of the sulfur deposited to forest soils in throughfall at study sites in a forested area of East Tennessee comes from atmospheric sources. They also showed that forest canopy throughfall has significantly higher concentrations of sulfur than rainfall that is not intercepted by the trees. The *Nature* paper states that "the dominant source of sulfate in net throughfall at these sites is wash-off of dry-deposited particle sulfate and of sulfur dioxide that is oxidized to sulfate within the forest canopy following deposition."

The sulfur-35 tracer was used to determine that leaching of internal sulfur contributed only a small portion (2–14%) of the sulfur enrichment in throughfall beneath eight different trees. This result contradicts a British study that identified leaching from foliage as the primary source of sulfate enrichment in throughfall in a pineforest The ORNL study was conducted on two deciduous tree species (red maple and yellow poplar) and one conifer (loblolly pine). The study region is exposed to sulfur emissions from several nearby coal-fired power plants.

According to Lindberg and Garten, the finding has important implications for understanding and predicting the effects of acid rain and atmospheric deposition on trees, soils, and lakes and for testing atmospheric transport models and emission control strategies. They conclude that measurements of sulfate in throughfall below forest canopies can indicate trends in atmospheric sulfur inputs to forested ecosystems in industrialized areas.

The research was performed as part of the U.S. National Acid Precipitation Assessment Program and of the Integrated Forest Study, sponsored by the Electric Power Research Institute.

The Walker Branch Watershed Project: 20-Year Results

By Jerry Elwood, Dale Johnson, Robert Van Hook, and Webb Van Winkle

In 1967 ORNL's Environmental Sciences Division (ESD) initiated the Walker Branch Watershed Project on the Oak Ridge Reservation. The project, which had been funded for 20 years by the Atomic Energy Commission and its successor agency, the Department of Energy, focused on three principal objectives:

- developing baseline data on an unpolluted forested watershed ecosystem,
- expanding our knowledge about chemical losses and movements of nutrients and contaminants (cycling) and water (hydrologic transport) in natural forested ecosystems, and
- providing a basis for the construction of mathematical models for simulating the behavior of forested watersheds and predicting the effects of human activities on such ecosystems.

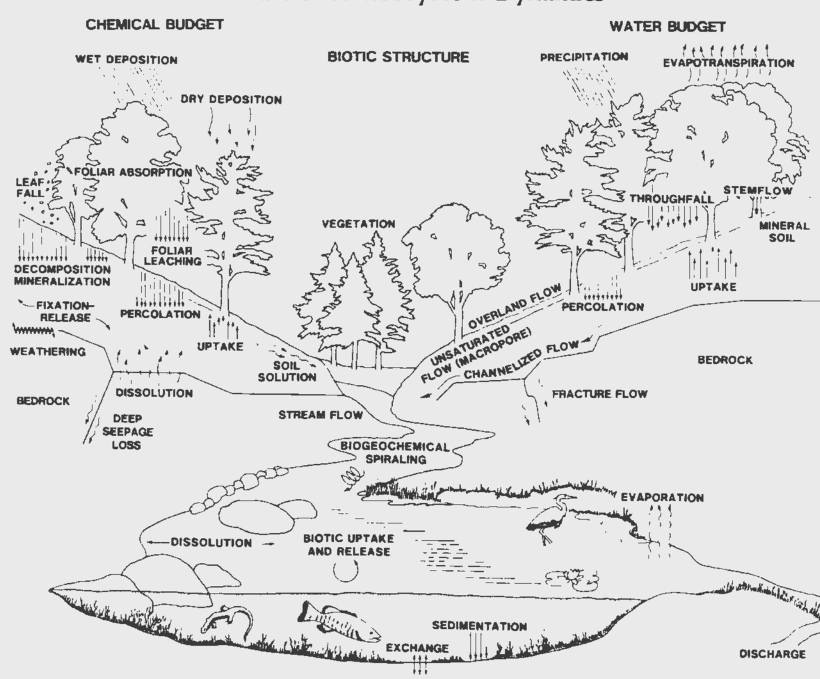
Scope of the Study

In 1969, when ESD was chosen to lead the International Biological Program's Eastern Deciduous Forest Biome Project (see Auerbach's article on p. 62), the Walker Branch Watershed was selected as one of several sites for intensive research on nutrient cycling and biological productivity. This work, supported by the National Science Foundation (NSF) for four years, coupled intensive process-level research on primary productivity, decomposition, and below-ground biological processes with ongoing DOE-supported research characterizing the geology, soils, hydrology, and cycles of major nutrients on the watershed.

With funding from the NSF RANN (Research Applied to National Needs) Program, we began work on trace-element cycling in Walker Branch Watershed in 1974, using our already extensive data base on hydrology, soils, vegetation, and meteorology. We developed "budgets" for cadmium, lead, and zinc (estimates of quantities entering and leaving the watershed), significantly increasing our understanding of the cycling of these elements in the Walker Branch Watershed ecosystems.

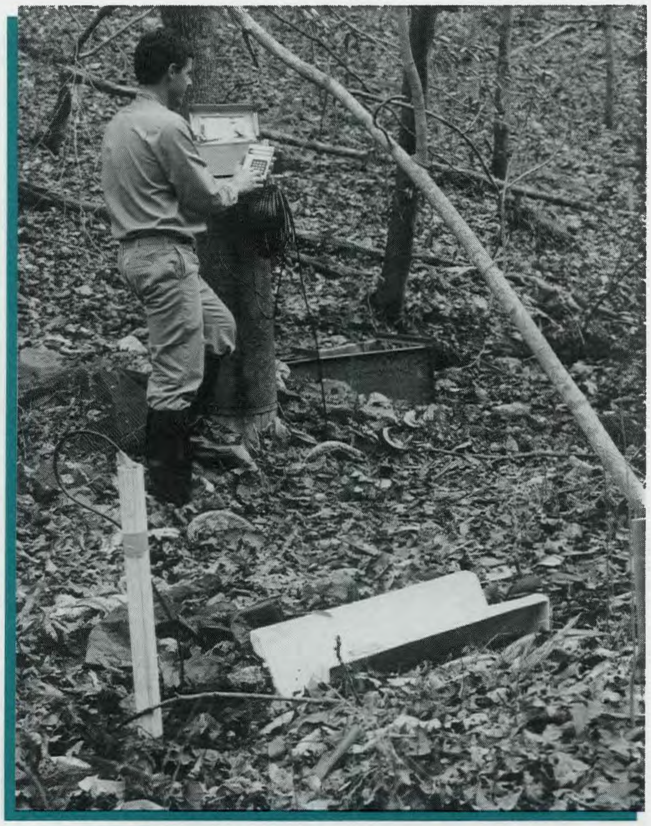
Our basic biogeochemical cycling research was also supplemented in 1978 by two major new projects. The first, funded by the NSF Ecosystem Studies Program, was the experimental verification of the spatially dependent cycling or

Watershed Ecosystem Dynamics



This longest-running U.S. ecological survey provides baseline data for understanding and predicting changes in forest ecosystems.

In this aerial photograph (published on the cover of *Science*), a meteorological tower extends up through the Walker Branch Watershed forest canopy. The tower is used to study forest meteorology and atmospheric deposition.



Patrick Mulholland retrieves data from remote, battery-powered instruments that continuously record water flow through flumes placed on springs discharging to Walker Branch, a small stream.

spiraling of nutrients phosphorus (P) and nitrogen (N) in streams. This work provided quantitative information on the efficiency with which a stream ecosystem utilizes the available supply of a nutrient relative to its downstream flux (i.e., how much of each nutrient is used by the stream's organisms, and how much washes downstream). The second project, sponsored by the Electric Power Research Institute, addressed atmospheric deposition and the effects of acidic precipitation on forests. This study showed that the amount of sulfur compounds leached from soil soaked by acid rain depends heavily on the amount of dry deposition of sulfates and the ability of the soil to retain them.

Comprehensive Results

The Walker Branch Watershed Project is one of only a few long-term intensive ecosystem studies done in the United States. Numerous collaborators from universities, national and international institutes, and federal agencies (particularly the National Oceanic and Atmospheric Administration's Atmospheric Turbulence and Diffusion Division in Oak Ridge) have been involved in the project. These two decades of research are summarized in a recently published book presenting a comprehensive site description of Walker Branch Watershed; in-depth analyses of its biogeochemical cycling, carbon cycling and productivity, water balances, forest micrometeorology, and stream spiraling; and mathematical simulation modeling.

Our most significant scientific accomplishments in this research have been in documenting the importance of dry deposition in atmosphere–forest canopy interactions; defining the role of soil macropores in hydrologic and contaminant transport; analyzing the biogeochemical cycles of carbon, nitrogen, sulfur, major cations (e.g., calcium and magnesium), and several trace elements; and establishing long-term data bases on atmospheric deposition, hydrology, stream flow, biomass, and soils. Our atmospheric deposition research has helped prove the significance of dry deposition in the overall atmospheric input of elements (e.g, sulfur) to the watershed; for example, our studies have shown that dry deposition represents 50% of the overall input.

Detailed hydrology studies conducted by Bob Luxmoore, Glen Wilson, and Phil Jardine have identified the important role of large soil pores (macropores) in subsurface water transport, leading to the development of additional research activities and improved simulation models for below-ground water transport. Transport of solutes from the hillslope to lower landscape positions on the watershed during storms has been found to be largely controlled by preferential flow through macropores. Thus, during storms, the mobility of highly reactive constituents in soil, such as dissolved organic carbon, increases significantly. Slow vertical migration through smaller pores of the soil matrix is also a significant transport process for some solutes during drier periods.

The development of detailed biogeochemical cycles for various elements and cations has also contributed to our understanding of the basic ecological processes governing cycling and the response of these cycles to human stress (e.g., increased acidic pollution resulting from industrialization). The long-term trends, which become obvious only in data sets such as ours that have been maintained for a long time, have highlighted changes in species composition and in the biogeochemical cycles of various elements in the watershed. Our long-term data also provide baselines for assessing future changes.

Research Applications

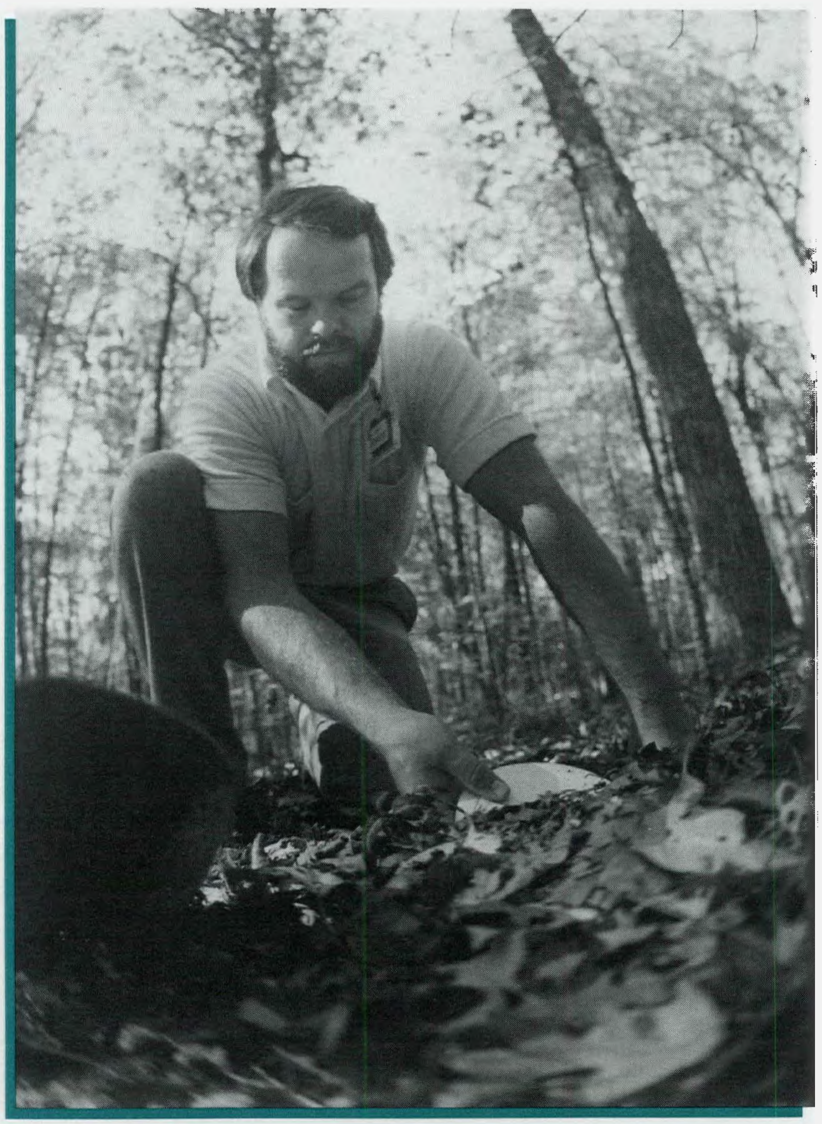
We have applied the results of Walker Branch Watershed research in two ways. First, our understanding of the fundamental behavior of forest ecosystems developed over the course of our research has allowed us to answer specific questions concerning the effects of stress on forested landscapes. As problems arise on adjacent watersheds or on forested landscapes elsewhere in the eastern deciduous forest (e.g., air pollution stress and heavy-metal input), our knowledge of basic processes derived from this research guides our investigations.

Second, the combination of long-term data sets for ecosystem changes and the basic information gained on specific ecological processes has provided a unique tool for extrapolating Walker Branch Watershed results to all eastern deciduous forests. For example, the sensitivity of various soils in the eastern United States to acidification from deposition has been determined and mapped, based on our research results on ion mobility in Walker Branch Watershed soils, coupled with soil measurements obtained from other sites in the region. The long-term vegetation data for Walker

Jerry Elwood and Amy Rosemond (graduate student from Vanderbilt) examine samples of algae growing on rocks from experimental stream channels next to a Walker Branch Watershed stream. Their goal is to determine whether nutrients or grazing invertebrates limit algae growth.

Branch Watershed, along with the forest growth simulation models developed at ORNL, are useful in assessing the effects on forests of human stresses such as air pollution.

As research on Walker Branch Watershed enters its third decade, the scope continues to expand. We are initiating new studies to investigate the biochemical cycling and transport of selected nutrients along the upslope-to-downslope axis of Walker Branch Watershed on different space and time scales. We will focus on developing a quantitative description of carbon (C), N, and P fluxes and chemical transformations. The research is needed because the transport, fate, and bioavailability of these nutrients provide sensitive indices of the dynamics of watershed systems and because the cycling and transport of these nutrients may be coupled directly or indirectly with the transport, fate, and effects of some energy-derived contaminants, such as toxic trace metals and organic chemicals.



Dale Johnson inserts a lysimeter into the soil on Walker Branch Watershed. Lysimeters are used to collect soil water for chemical analysis of nutrients and environmental contaminants.

The major theme of our current work is quantification of hydrologic, chemical, and biotic linkages between components (e.g., canopy, litter, mineral soil, and groundwater) and between subsystems (e.g., upslope, riparian, and stream) of the watershed, particularly linkages that transcend traditional ecosystem boundaries, such as those between ridge tops, side slopes, and valley bottoms and between terrestrial and aquatic systems. We plan to quantify the extent to which, and conditions under which, biogeochemical processes in upslope and upstream systems influence the magnitude and forms of C, N, and P fluxes (and, in turn, biological responses) in downslope and downstream systems. Because most of our past studies on the watershed have been conducted within individual components and subsystems of the Walker Branch Watershed, connections between these components and subsystems have not received as much attention. Thus, our conclusions have been too limited to be extrapolated to other systems.

Future Focus

During the next decade, we plan to continue this research, addressing questions about how and when watershed components and subsystems are functionally linked. We anticipate that by expanding the spatial boundaries of our research, we will facilitate conceptual advances in watershed science. Hierarchical analysis is one approach we plan to use in identifying the hydrologic pathways and biogeochemical processes most important in controlling the chemical forms and fluxes of C, N, and P on various space scales (e.g., on a 0.5-ha hillslope near the ridge line vs in the perennial stream draining the 40-ha western section of Walker Branch Watershed) and time scales (e.g., storm events vs base flow).

We initiated the Walker Branch Watershed Project with the goal of understanding the basic biological, chemical, and physical processes and their interactions that govern the cycling and transport of materials in forested watershed ecosystems. Although the project began years before public concerns arose about acidic precipitation, increases in atmospheric carbon dioxide, emissions of trace elements from industrial sources, and shallow land burial of hazardous wastes, what we have learned from these studies about watershed ecosystem dynamics has provided the basis for quickly responding to such problems. Our results have demonstrated the value of long-term basic research in providing the knowledge needed for understanding and resolving these and other serious environmental problems that may arise. ornl

Jerry W. Elwood, a senior research staff member of the Environmental Sciences Division, began working at ORNL in 1968, after earning his Ph.D. degree in ecology from the University of Minnesota. He is a fellow of the American Association for the Advancement of Science.

Webb Van Winkle, head of ESD's Ecosystem Studies Section, earned his Ph.D. in zoology from Rutgers University and came to ORNL in 1972. He is a fellow of the American Association for the Advancement of Science.

Dale W. Johnson, an ESD senior research staff member, came to ORNL in 1977, two years after obtaining his Ph.D. degree in forest soils from the University of Washington. He has received ESD's Scientific Achievement Award, two Martin Marietta Energy Systems Publications Awards and one Energy Systems Technical Achievement Award.

Robert I. Van Hook's biographical sketch is given on p. 80.

Patterns of Landscape Disturbance

By Robert H. Gardner

The spatial mosaics formed by forests, fields, lakes, and roads make different landscapes easily recognizable. Thus, by looking at an aerial photograph, a person can easily distinguish the landscape of Virginia from that of nearby Pennsylvania. Less apparent to the casual observer are the materials and organisms that move between these landscape elements. Because disturbances such as urban growth, introduced pests, and fire can dramatically alter landscapes, ecologists are actively studying the interaction between the various patterns of the mosaics and the spread of disturbance.

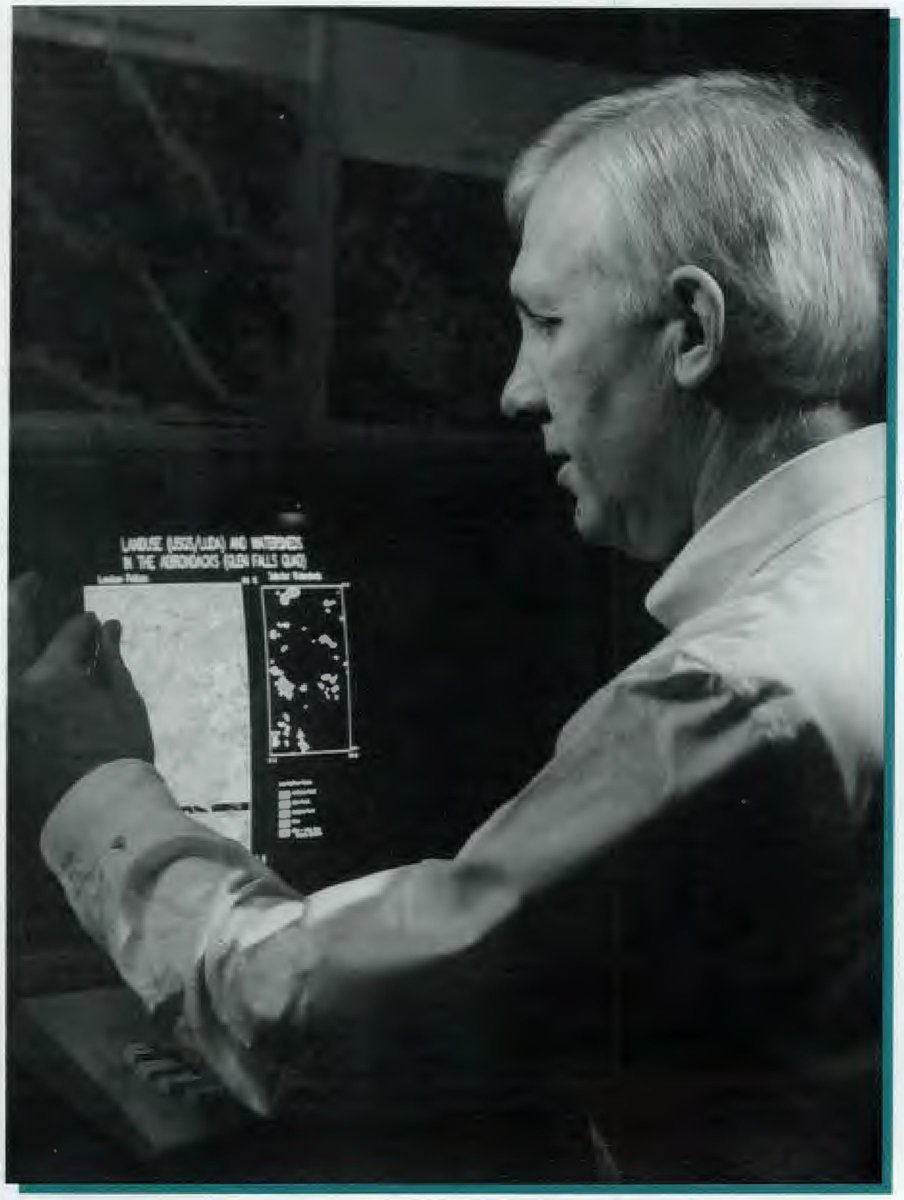
Because landscape studies are both difficult and expensive to conduct in the field, researchers are turning to simple computer models as an aid in predicting the effects that disturbances have on the landscape. Such models generate landscape patterns with which observed patterns can be compared. By noting deviations between the predicted and observed patterns, researchers can identify the relative importance of the variables involved.

Generating the Patterns

We have recently adapted methods based on the theory of spatial arrangement known as percolation theory (developed by D. Stauffer at Cologne University) to generate two-dimensional gridded patterns that are graphically similar to maps of landscapes. Two-dimensional percolation maps are formed by randomly assigning to each grid site a particular landscape element, or habitat (often either forest or grassland), and establishing the probability, p, for that habitat's occurrence. The value of p is usually determined from the fraction of the total landscape that is covered by that habitat. For example, if we want to generate a landscape that is 50% forest, we set p at 0.5.

According to percolation theory, clusters form

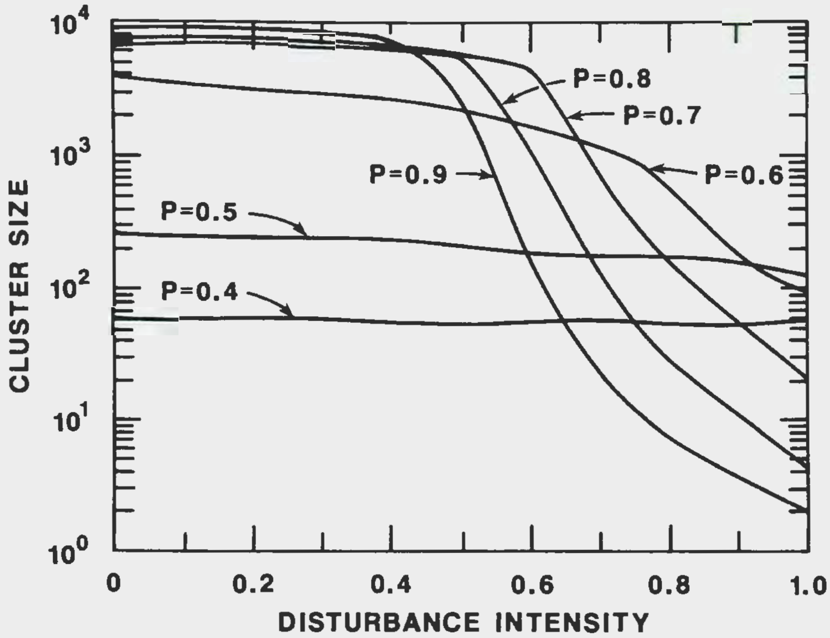
This aerial photograph of ORNL's Fuel Recycle Division shows the surrounding landscape mosaic—forest, field, lake, and hills.



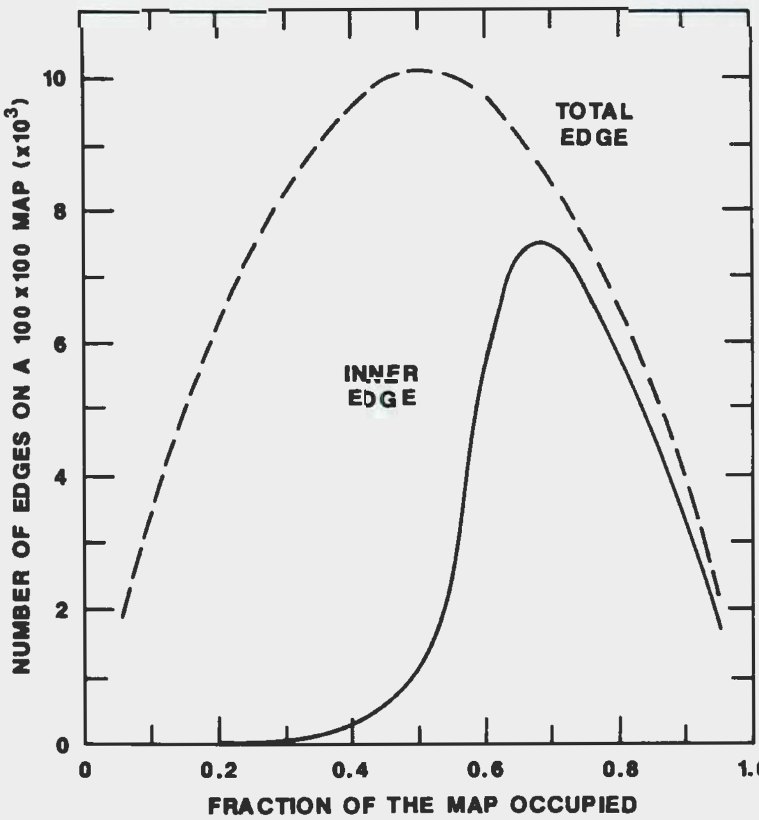
Bob Gardner views a computerized landscape pattern for the Adirondack Mountains. ORNL scientists have analyzed data on land use and land cover from the U.S. Geological Survey's LUDA data base. Computer images generated from this data base allow the spatial patterns of habitat types to be verified.

when adjacent grid sites are occupied by the same landscape element. Computer simulations show that, when p is small, few clusters larger than three sites are formed; as p increases, the average size of the clusters increases (see top figure, p. 106). Furthermore, as p approaches 0.6, adjacent clusters coalesce to form larger clusters that span the map or "percolate" from one side of the map to the other. The "percolation" threshold, p_c, for very large maps ($>10^6$ sites) has been more precisely determined as $\sim p = 0.5928$.

The frequency and arrangement of landscape elements are important for many ecological processes. For example, deer inhabit the edges of forest clusters, where they have access to bordering fields. The movement of nutrients, the spread of fire, and the invasion of pests are also related to the degree of clustering. In a computer model, the degree of clustering can be characterized by the relationship between the number of inner edges and the number of outer edges (see lower figure on p. 106), with "edge" defined as a cluster surface that is adjacent to a grid site covered by a different landscape element. "Outer" edges lie along the outside of the cluster; "inner" edges are formed when a cluster completely encloses a grid site with a different landscape habitat, creating an inner gap, or patch (e.g., an open patch of grassland in the middle of a forest). The number of inner edges declines rapidly as p is reduced below p_c. As cluster size declines, gaps within a cluster tend to widen and become part of the external edge of the cluster.



These percolation-theory results indicate that the effects of habitat disturbance become obvious when a given habitat occupies ~60% of the landscape area (p = 0.6). Above this critical value, disturbance intensity becomes increasingly important.



Number of edges observed on a randomly generated map as a function of p, a particular habitat's occurrence on the map.

When p falls below 0.4, very few clusters have internal patches to form inner edges.

Because individual species respond differently to landscape characteristics, it is reasonable to expect different spatial distributions of species as a function of p. Thus, it may be possible to maintain optimally structured landscapes or landscape "patterns" having the desirable frequencies of size, shape, and edges of patches to ensure the presence of desirable species. Current results (see upper left figure) indicate that disturbance of habitat might be most critical near $p = 0.6$.

Modeling the Disturbances

Simple computer models can use the landscape patterns generated from percolation theory to simulate the spread and effect of disturbances. In the models, a landscape can be characterized in terms of habitat types that are susceptible to disturbance (e.g., pine forests are more susceptible to bark beetle infestations than are oak-hickory forests). The spread of disturbances within the susceptible habitat can then be specified by the frequency and intensity of disturbance (the ability of a disturbance to affect adjacent sites). Simulation results indicate that the spread of a disturbance is qualitatively different when the probability, p, of the landscape occupied by susceptible habitat is either greater or less than p_c. If p is less than p_c, clusters tend to be fragmented. The spread of a disturbance is constrained by this fragmented spatial pattern, and the sizes and numbers of clusters are not substantially affected by the intensity of the disturbance. However, disturbance frequency is important to the persistence of a rare habitat. If p is greater than p_c, habitats tend to form continuous clusters; in addition, disturbance intensity becomes a more important factor than frequency, because a disturbance can spread from site to site within the cluster even when the disturbance frequency is relatively low. At high values of p, the habitat appears very susceptible to fragmentation by disturbances of only moderate intensity.

Goal Is Predictive Theory

We have used percolation theory to suggest that a few parameters describing spatial heterogeneity and the spread of disturbance can aid in estimating the effects that disturbances have on the landscape. However, unlike the structure of the computer-generated patterns, the structure of real landscapes is not random, for the close association of sites covered by similar habitat occurs more often than expected if randomness is assumed (e.g., the clustering of forested sites is not dictated by chance alone). In addition, the effects of disturbances might be observed at lower intensities in landscapes having a high degree of clustering. Nevertheless, we believe that our approach, which combines the power of computer simulations with the insights of new empirical studies, may lead to a useful predictive theory about the spread of ecological disturbance. ornl

Robert H. Gardner is a senior research scientist in the Environmental Sciences Division. He came to ORNL in 1974 after receiving a Ph.D. degree in ecology from North Carolina State University. Assisting him with the landscape research are Monica G. Turner, Virginia H. Dale, and Robert V. O'Neill.

Energy and Natural Resources: Problems and Opportunities

By David S. Shriner

For scientists in the Environmental Sciences Division (ESD), issues related to energy and natural resources are frequently inseparable. Whether the issues are the impacts of energy technologies on natural resources or the promise that conventional wood resources hold in meeting national goals for domestic energy production, they fall within the scope of ESD research.

Understanding the relationships between energy production (e.g., generating electricity by fossil fuel combustion) and natural resources (e.g., forests and lakes) requires a combination of expertise and experience that ESD can provide. Our studies on atmosphere-landscape interactions and land-water interactions have ranged from examinations of the sulfur and trace metal budgets at Walker Branch Watershed to analysis of the global carbon cycle and the effect of global carbon dioxide on climate change.

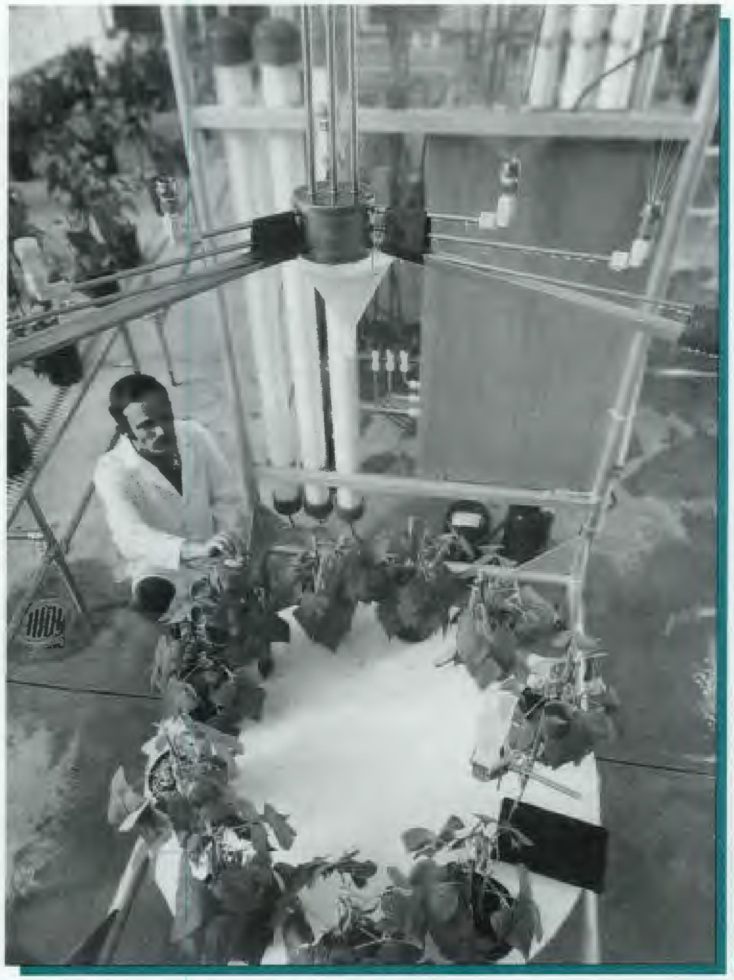
ESD scientists have also investigated the effects of acid rain, ozone, and carbon dioxide on plant physiology and growth and developed models to study forest succession under changing pollution or climate scenarios. By combining data from our ecological studies with computerized landscape patterns, ESD scientists can provide regional and national assessments of the potential impacts that changes in energy technology or energy use will have on forest, agricultural, and water resources.

This 1976 photograph shows Ron McConathy (left) and Sandy McLaughlin collecting and examining leaves for studies to determine the effects of atmospheric deposition on forests.

Acid rain, the global carbon cycle, and wood energy crops are ESD concerns.

The ESD-managed DOE Global Carbon Cycle Research Program is working to characterize the global carbon cycle as a means of predicting future concentrations of atmospheric carbon dioxide and the potential impacts of increased fossil fuel combustion. There are many uncertainties about the size of major carbon dioxide reservoirs (e.g., forests and oceans) and the types and sizes of transfers within the global carbon cycle. Increased releases of carbon dioxide to the atmosphere as a result of escalated fossil fuel combustion may have significant future impacts on the earth's climate and natural resources. Such carbon-dioxide-induced changes in climate and vegetation would affect many important decisions about the use of land and other natural resources. One aspect of the ESD research in this area is seeking to define an appropriate means for ranking these resource issues by their importance to society.

Another ESD project relating to natural resources is the Short Rotation Woody Crops Program, also managed by ESD for DOE. The program's goal is to develop wood-energy crops to replace petroleum-based fuels. If such a new biomass-based energy industry is to be sustained simultaneously with other forest product industries, wood must be produced faster and in greater quantities. Our studies have shown that intensive culture of hardwood forest species can amply meet these needs. Researchers are working to develop fast-growing species and cultural practices that will offset the higher costs of intensive culture by increasing the productivity of marginal to good agricultural land. ornl



In 1978, Bill Selvidge and others studied the effects of simulated acid rain (having a precisely measured acidity) on the growth of a variety of crop species.

David S. Shriner is leader of the Regional Resources Group in ESD's Environmental Analyses Section. He came to ORNL in 1974. He has a Ph.D. degree in plant pathology from North Carolina State University. Additional contributors to this article were Robert I. Van Hook and Michael P. Farrell.

Complexities of Acidic Deposition

By S. B. McLaughlin, J. W. Elwood, R. B. Cook, D. W. Johnson, S. E. Lindberg, R. J. Olson, and R. S. Turner

The deposition of strongly acidic sulfur and nitrogen oxides resulting from combustion of fossil fuels has been blamed for the decline and death of forest trees, reduced soil fertility, destruction of fish populations in lakes and streams, deterioration of surface details of marble monuments, and even impaired human respiratory function. However, the fate and effects of the acidic substances in the gases, particles, and precipitation that constitute "acidic deposition" are still very controversial issues, and the cause-and-effect linkages demonstrated to date have not yet proven sufficiently compelling to force legislative control of industrial emissions in the United States. The challenge and national urgency of obtaining evidence to determine scientifically whether strict emission controls would benefit the environment has drawn many ORNL scientists into national research programs on the environmental effects of acidic deposition.

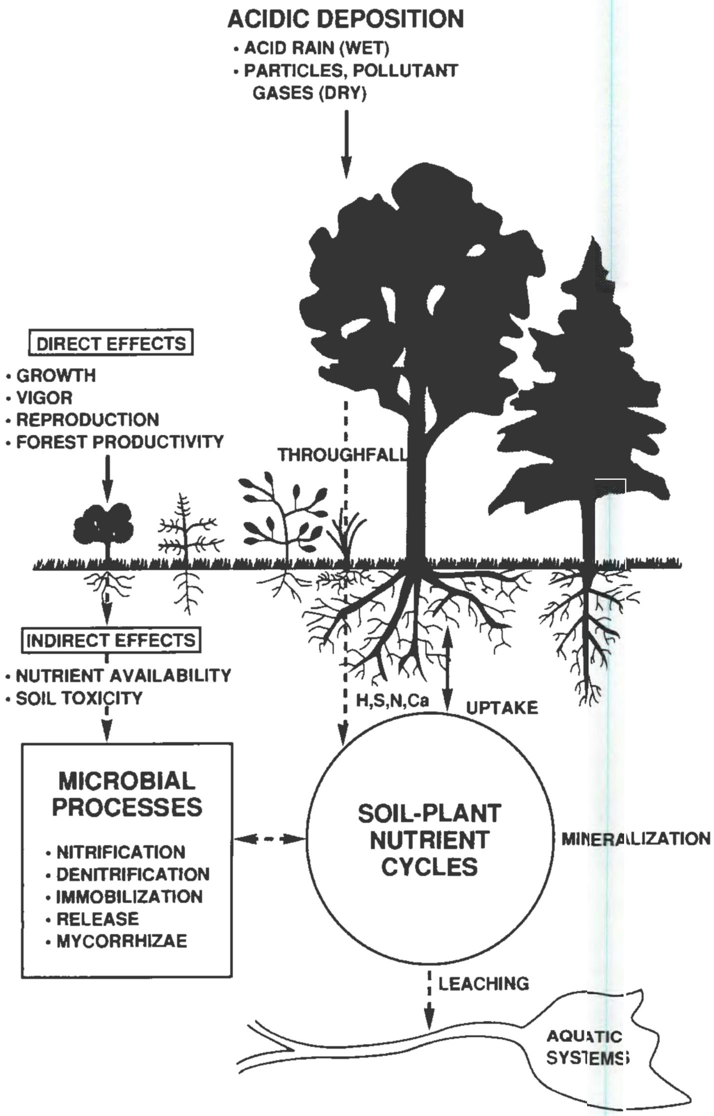
Evolution of the Issue

Acidic deposition first became an environmental issue of public interest in 1972, when Sweden reported to the United Nations that European air pollution was threatening living organisms in Scandinavian streams, soils, and forests. Since that time, the environmental impact of acidic deposition has become a hotly debated, international issue. Why the concern? What are the major points of controversy? And what role has ORNL played in resolving the principal issues?

Concern about the effects of acidic deposition stems from several sources. First, changes in the chemistry of our precipitation, soils, and streams have been measured over large regions. These measurements indicate that inputs of acidity in and downwind of industrialized regions have increased substantially over expected normal background levels and that the atmospheric environment has been altered over areas extending hundreds of miles from the centers of industrial activity. In the Oak Ridge area and in much of the northeastern United States, for example, the acidity of annual rainfall averages about 5 times the expected value based on the chemistry of unpolluted atmospheres. On Clingmans Dome in the nearby Great Smoky Mountains National Park (GSMNP), trees and soil are frequently exposed to cloud moisture that, on the average, is several times more acid than low-elevation rainfall in surrounding areas.

Coincident with changes in atmospheric chemistry have been widespread reports of deterioration in the environmental quality of forests, soils, and streams. According to some reports in the popular press, this ecological damage could be eliminated by immediately reducing the sulfur and nitrogen oxides released by fossil-fuel combustion. Unfortunately, the solution may not be so clear and simple.

Many uncertainties remain about the chemical and biological mechanisms responsible for the observed environmental changes attributed to increases in acidic deposition. The aquatic and terrestrial ecosystems at risk are enormously complex, and many processes interact in controlling the health of these systems as they respond to a wide array of natural environmental stresses. Separating the effects of human-induced (anthropogenic) stresses within this complex is, in fact, a very difficult task. In addition, because of the wide distribution of acidic deposition and the absence of long-term monitoring of environmental conditions in most areas, it is difficult to find suitable "control" areas to determine "normal" conditions with no acidic deposition. A final major complexity is that the removal of sulfur (and possibly nitrogen) from emissions will cost many billions of dollars, possibly leading to harmful economic, sociological, and environmental consequences. Because of the difficulty in providing direct proof linking emissions to widespread environmental degradation and because of the high costs of



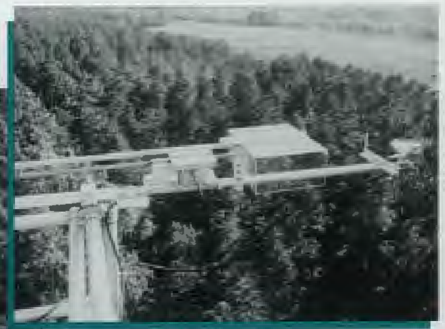
Major areas of concern for interactions of acidic deposition with forests include both direct effects (contact with plant surfaces) and indirect effects (changes in soil chemical balances). Influences on aquatic systems are related to the vegetation and soil that mediate transport of acidity to streams and lakes.

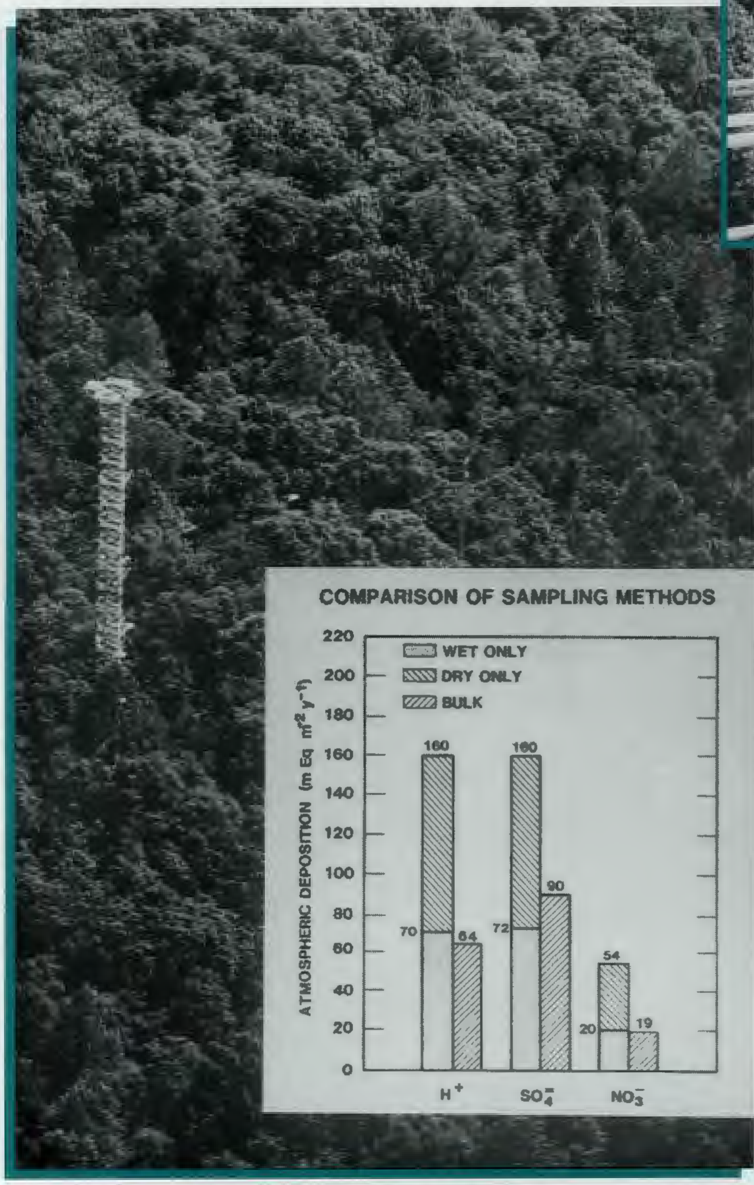
installing and maintaining control devices, special interest groups representing industry have strongly opposed emission control. Thus, a decision to reduce emissions must be based on scientifically (and legally) defensible evidence indicating the regions that would benefit from emission reduction, the types of pollutants requiring control, and the maximum permissible limit for emissions.

Since the mid-1970s, ORNL scientists have addressed and helped to resolve many of the uncertainties linked to acidic deposition issues. Our pioneering research projects have grown from small-scale monitoring of rain chemistry and laboratory exposure of greenhouse-grown crop plants to simulated acid rain (initiated by Dave Shriner and others in 1975) to regional-scale projects covering major soil and vegetation gradients in the eastern United States. Our research has reflected a belief that such a complex issue should be approached at many scales to understand both the larger-scale patterns of environmental response and the mechanisms underlying those responses. Our patterns-vs-mechanisms approach has been useful for investigating the chemistry and distribution of acidic deposition and characterizing its effects on both terrestrial and aquatic ecosystems.

Chemistry of Acidic Deposition

Detailed measurements by Steve Lindberg of ORNL's Environmental Sciences Division (ESD) have dramatically increased our understanding of the nature of acidic deposition and the total amount and form of acidity delivered to the landscape. It was previously thought that wet deposition of acidity as rain or snow was the dominant source of acid deposition. However, research by Lindberg and colleagues has shown that dry deposition and cloud-water deposition, particularly on the foliage of high-elevation forests, may be equally or more important than wet deposition. Dry deposition includes the scavenging of gases and of particles blown by wind onto tree leaves and needles. Cloudwater deposition occurs when forests are exposed to wind-driven clouds that deliver acidic chemicals dissolved in the cloud droplets. Dry and cloud-water deposition have been ignored in most previous studies





Sampling of chemical inputs to forest canopies using ORNL-designed collectors (top inset) has shown the importance of dry and cloud-droplet deposition to forested landscapes.

because of the difficulty in collecting information about these processes. In early studies, measurements of material deposited in an open sampling bucket provided estimates of total acid inputs to the forest. By developing field, laboratory, and modeling techniques, Lindberg and his co-workers have shown that the traditional sampling techniques seriously underestimated the total input of acids to forested landscapes.

Based on research at ORNL's Walker Branch Watershed, supported by the Department of Energy, and on Clingmans Dome, supported by the Electric Power Research Institute (EPRI), this group has found that from 40 to 70% of sulfur and nitric acids deposited on lower-elevation forests is in the form of dry deposition. At Clingmans Dome, where cloud moisture is more significant, the amount of cloud- and dry-deposited acidity is 3 to 6 times the amount deposited as rain or snow. Such measurements are crucial to understanding the routes by which acidity reaches forested ecosystems.

Analysis of Regional Trends

Two versatile and powerful regional data base systems, the Geoecology Data Base and ADDNET, an acronym for Acid Deposition Data Network, have been assembled by Dick Olson and co-workers for examining the spatial patterns of acidic deposition with respect to (1) the contributors to that acidity, such as industrial emissions, and (2) chemical, physical, and biological features of the landscape that ultimately determine the extent and nature of resources at risk. The Geoecology Data Base, which has been designed to examine a wide variety of national

These maps, developed from ORNL's Geoecology Data Base, show distribution patterns for wet acidic deposition, ozone concentration, and principal forest types in the eastern United States for 1980 to 1982. The inset graph shows historical patterns for releases of industrial pollutants up to that time.

environmental problems, contains extensive national environmental data for over 1000 variables for each of 3071 county units in the conterminous United States. The ADDNET has been assembled specifically to supply data needed by the National Acid Precipitation Assessment Program in evaluating regional-scale impacts associated with acid deposition. It contains extensive national data sets covering emissions, air quality, deposition, water quality, forestry, and soils and is frequently updated to reflect new indicators of sensitivity in the biological systems it is designed to assess.

Through ADDNET and other regional data bases on the chemical and biological quality of the nation's streams and lakes, ORNL has played a key support role in the national Aquatic Effects Research Program of the Environmental Protection Agency (EPA). Paul Kanciruk and Mike Sale lead a data base management team that has analyzed survey data from about 2500 lakes and 1000 streams and defined for the first time the spatial extent of acidic and acid-sensitive waters in the United States. The data base management and analysis skills of Kanciruk and his co-workers were recognized by EPA, which awarded this team the Bronze Medal of Commendation.

In related work aimed at identifying the key soil and geological features contributing to the distribution of sensitive lakes and streams, ORNL geochemist Robb Turner has used the information from the aquatic surveys to classify 180 watersheds across the region, based on their potential to contribute acidity to these aquatic systems. Information on soils, vegetation, geology, pollutant deposition, and land use data were incorporated into an integrated data base used to develop

Donna Genung collects a sample of benthic invertebrates from a stream in the Great Smoky Mountains National Park This study was part of a larger project supported by EPRI for examining the chemistry and ecology of acidic and nonacidic streams.

empirical watershed models designed to predict the dynamics of watershed responses to acid inputs over time. Other models are being used to forecast changes in water quality for various pollutant levels. This work, for which Turner was awarded an EPA commendation, will be a key component of the 1990 National Acid Precipitation Program assessment of the potential future effects of acid deposition on stream and lake water quality.

Effects on Aquatic Systems

Two effects of acidic deposition on aquatic life have aroused concern: (1) acidity itself endangers stream life, and (2) aluminum, released from soil to streams by acidic deposition, is toxic to fish.

Most fish do not thrive at pH (acidity) values below 5.5, which is 10 to 50 times less acid than current rainfall (pH 4.0–4.5). Where the soils are naturally very acid, or where anthropogenic acidification has reduced natural buffering of acid rainfall, much of this acidity passes directly through the soils, releasing aluminum and other potentially toxic metals bound to the soil and carrying them into surface waters. EPA's random survey of streams in the eastern United States showed that the acid-neutralizing capacity of streams in the southern Appalachian region, including the GSMNP, is among the lowest of any of the regions sampled. ORNL surveys have shown that a few streams in the region are also acidic during baseflow. More intensive studies of these streams have shown that sulfate and nitrate account for their acidity. Although the source of acidity in these streams is not yet determined, all acidic streams in the region drain watersheds having internal sources of nitrogen and sulfur, suggesting that atmospheric deposition may not be their primary source of acidity. These studies, funded by EPRI, were conducted by Jerry Elwood, Ralph Turner, Pat Mulholland, Tony Palumbo, Mary Anne Bogle, and Bob Cook.

In another EPRI-funded study, ESD staff have examined the water chemistry and ecology of acidic and nonacidic streams in the GSMNP in Tennessee and North Carolina and in the Adirondack Mountains of New York. Although watershed sources of sulfate and nitrate may contribute to the strong acidity of GSMNP streams, the chemistry of these streams is similar to that of acidic streams in the Adirondacks, which were apparently acidified solely by atmospheric deposition. Comparative studies of biological communities and processes in the acidic and circumneutral streams of these areas indicate that acidification of stream water has adversely affected almost all biological components at all levels, including bacteria, algae, invertebrates, and fish. In the more acidic streams, changes are noted not only in the diversity of species that populate the streams but also in the structure of these communities that depend on energy producer–consumer interrelationships that balance system trophic levels. For example, both fish and the aquatic insects on which they feed declined in population as stream acidity increased. The most acidic streams lacked fish entirely, and the slightly less-acidic streams supported only acid-tolerant species such as brook trout.

One of the important challenges for ESD researchers studying areas such as GSMNP, where acidic geological substrates occur naturally, is to determine the relative contributions of natural and anthropogenic sources of acidity reaching streams. Bob Cook and Ernie Bondietti are addressing this issue in the GSMNP using sulfur-35, a radioisotope that helps them differentiate between recent and longer-term movements of sulfur from soils into streams. Because sulfur-35 is produced naturally in the atmosphere, is deposited during precipitation, and has a half-life of only 87 days, it is being used in these studies as a short-term biogeochemical tracer.

Preliminary results of studies on Walker Camp Prong, one of the GSMNP streams studied by Elwood and colleagues, have shown that most of the sulfur in stream water during high-flow periods caused by a storm event could not be attributed to sulfur deposited during that storm. Atmospheric sulfur-35 entering the watershed during storm events is retained there, presumably in the soil. The sulfur being exported from these watersheds during storms is either derived from bedrock or atmospherically derived sulfur that has remained in the watershed long enough to have lost an appreciable amount of the short-lived sulfur-35 through radioactive decay. Based on these preliminary measurements, it appears that sulfur-35 is a useful tracer for examining both the short-term dynamics of sulfur cycling in larger watersheds and the role of atmospheric sulfur deposition in stream acidification during storms.

Effects on Terrestrial Systems

Concern about the effects of acid deposition on terrestrial systems has focused principally on the growth of forest trees and the stability of forest ecosystems, including soils. From a national perspective, the two forest types showing the most extensive evidence of reduced growth are the spruce and fir forests in the Northeast and at high elevations in the southern Appalachian mountains, where acid deposition is highest, and the southeastern pine forests, where ozone concentrations are highest. The spatial distribution of forest types with respect to these two regional pollutants can be more readily visualized with the aid of computer-generated maps from ORNL environmental data bases shown on p. 113.

Soils. Soil chemical reactions play a pivotal role in determining the extent to which acidity is neutralized in passing through the soil profile to streams; thus, they constitute a key linkage between terrestrial and aquatic systems. However, the neutralizing effect of forest soils can occur at the expense of critical pools of nutrients, such as calcium and magnesium, which are essential to sustaining forest productivity. Dale Johnson, Robb Turner, and their ESD colleagues are playing key roles in our regional-scale research linking experimental and theoretical aspects of soil chemical responses to acidic deposition. Johnson has directed two regional-scale collaborative studies. The first compared relative soil nutrient losses resulting from various timber harvesting techniques with losses that occur naturally and as a consequence of acidic deposition. Anthropogenic inputs of acidity from fossil fuel combustion were found to approximately double the rates of natural leaching losses of calcium from the forest soils studied.

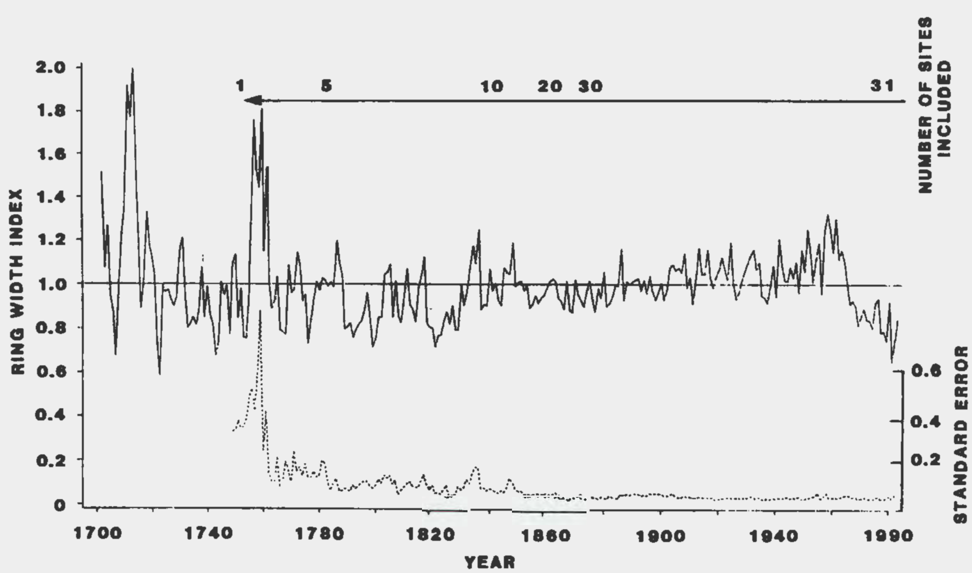
In their more recent work for the Integrated Forest Study funded by EPRI, Johnson and Lindberg have teamed with other investigators at seven sites in the eastern United States, one site in

Bryan Geerlings, a University of Birmingham student, worked with ESD staff to analyze the chemistry of a stream in the Smokies as part of an EPRI-sponsored study.

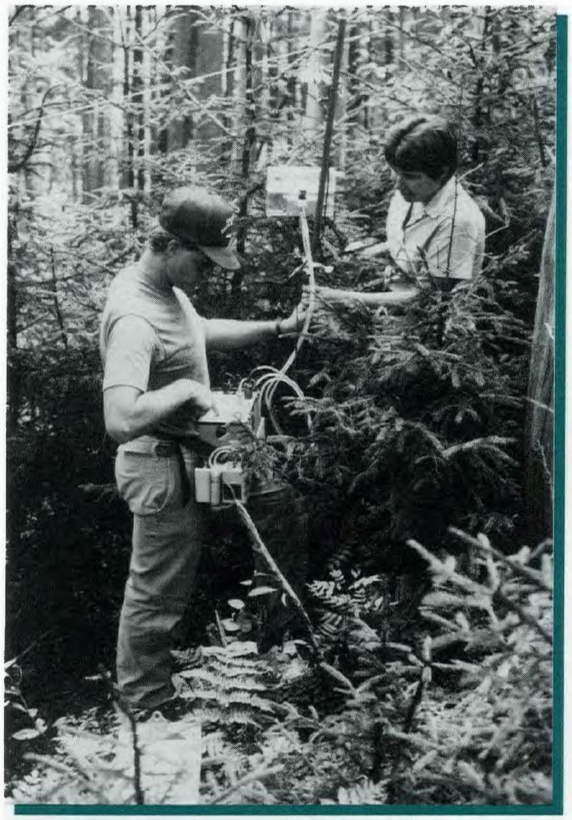
Bob Cook samples snow in the Smokies to determine the natural input of sulfur-35, which is useful in studying the dynamics of sulfur storage and release in a forested watershed.

the state of Washington, and one in Norway to examine the effects of sulfur and nitrogen on nutrient exchange both within the forest canopy and within the soil profiles. This research compares site differences in vegetation, soils, and levels of atmospheric deposition. One early important finding from this work is that, at high-elevation sites in the GSMNP, the high atmospheric inputs of sulfur and nitrogen oxides are particularly effective in releasing aluminum from the soil, making it available for transport to streams.

Forests. Widespread reports of forest death and decline in Europe (principally in the Federal Republic of Germany) and in the northeastern United States during the early 1980s touched off enormous controversy over whether to attribute the loss of forests to natural or human causes. Acid deposition has been in the forefront of this controversy, and ORNL scientists have contributed their fair share both to the controversy and to its resolution. In the first regional-scale study of forest growth trends in relationship to patterns of atmospheric pollutants, Sandy McLaughlin headed a study with T. J. Blasing, Linda Mann, and co-workers in ESD to examine annual growth data derived from tree cores of nearly 10,000 trees



ORNL measurements of annual tree-ring widths at 31 sites show that red spruce growth in the eastern United States has decreased sharply since 1960, an unprecedented departure from the long-term tree growth pattern.



Using a gas analyzer and computer, Chris Andersen and Pat Layton measure photosynthesis rates in red spruce foliage. They are part of an ORNL team studying reduced forest growth at high-elevation sites near Clingmans Dome.

representing over 30 species and 15 eastern states (see figure on previous page). Named FORAST (Forest Responses To Anthropogenic Stress), this EPA-sponsored exploratory project was designed to evaluate the possibility that atmospheric pollutants play a role in the regional patterns, timing, and magnitude of forest growth changes. The conclusion that atmospheric pollution was a plausible contributor to declines in forest growth generated tremendous controversy and criticism in 1984, when these results were first presented. Additional analyses of those original data and more recent data collected by ORNL scientists and other investigators have substantiated the original findings.

The year-to-year changes in the chemical composition of tree rings in relation to forest growth trends have also been analyzed. In 1984, Fred Baes and McLaughlin showed that the annual growth rings formed by trees reflect past changes in the trees' chemical environment, suggesting that this is a good indicator of changes in the levels and composition of past regional pollution. For example, old short-leaf pine trees growing in the GSMNP's Cades Cove reflected the acidifying influences of copper-smelting operations at Copper Hill—over 50 miles away. Both the intensification of these emissions in the late 1880s and the court-induced closure of the open-pit smelter were reflected in the rise and abrupt drop in iron levels found in the tree rings formed in those years.

Using tree-ring samples from the Cades Cove trees and from spruce, fir, and hemlock trees at various elevations in the GSMNP, we found that the more recent trends of decreasing forest growth corresponded to increasing levels of aluminum and decreasing levels of nutrient elements such as calcium in tree tissues. These findings are of interest because they suggest ways in which acid deposition may affect forest soils and forest growth. More recently, Ernie Bondietti has greatly refined the theoretical basis for interpreting these changes. He has found that increases in the aluminum-to-calcium ratios in tree rings of many spruce and fir trees growing at highly polluted, high-elevation sites in the GSMNP occurred about 40 years ago, when regional emissions of sulfur and nitrogen oxides increased in the Southeast. This finding suggests that, in calcium-poor soil, the aluminum made available to tree roots after acid deposition frees it from soil is taken up by trees in place of the nutrient calcium; as a result, trees are stunted or made less resistant to other stresses such as drought or insects. This work is a major advance in clarifying the critical role of acid deposition in calcium leaching and aluminum mobilization in eastern forests.

Tree physiology is also being studied to determine whether acidic deposition induces changes in tree function. ESD researchers are characterizing the physiology of red spruce trees in high-elevation sites in the GSMNP to determine the basis of recent reductions in growth rate at those sites. In addition, at their field research site on the ORNL reservation, they are studying the effects of acid rain, soil, and ozone interactions on the growth and physiology of loblolly pine.

Since its formation in 1980, the National Acid Precipitation Assessment Program has focused on addressing the many scientific uncertainties related to acidic deposition. At a national level, information is rapidly being collected to help resolve these uncertainties and determine whether and how emissions should be controlled. ORNL scientists have played key roles in this process and will continue to do so. ornl

Sandy McLaughlin is a senior research staff member of the Environmental Sciences Division, which he joined in 1974. A physiological ecologist, he has a Ph.D. degree from Duke University. He is recipient of the ESD Scientific Achievement Award, a Martin Marietta Energy Systems Technical Achievement Award, and an Energy Systems Publication Award.

Jerry W. Elwood, an ESD senior research staff member, received his Ph.D. degree in aquatic ecology from the University of Minnesota and joined ORNL in 1968.

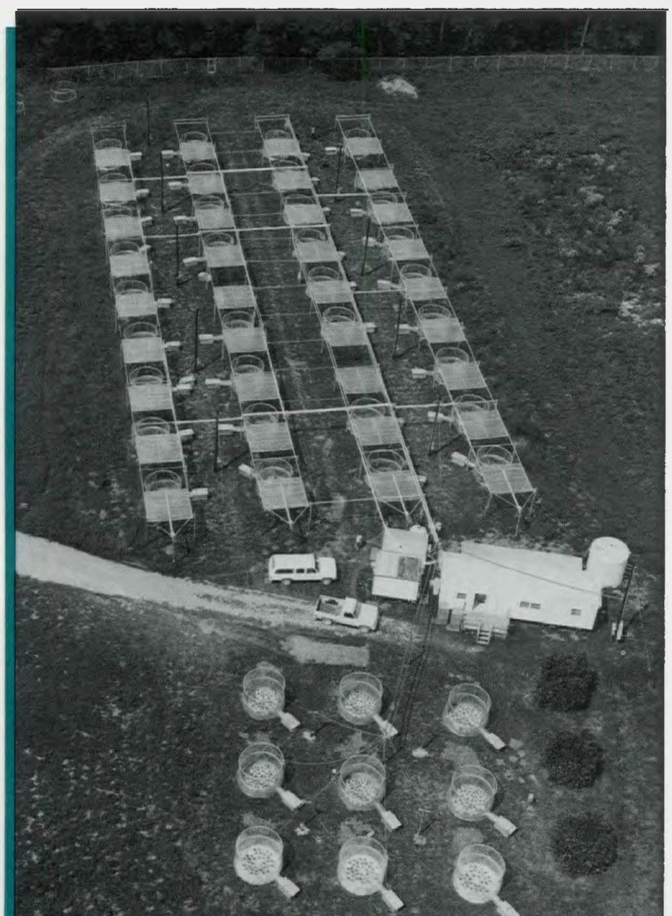
Robert B. Cook became an ESD research staff member in 1986. He has a Ph.D. degree in geochemistry from Lamont-Doherty Geological Observatory of Columbia University.

Dale W. Johnson, an ESD senior research staff member, came to ORNL in 1977, two years after obtaining his Ph.D. degree in forest soils from the University of Washington. He has received ESD's Scientific Achievement Award, two Martin Marietta Energy Systems Publications Awards, and an Energy Systems Technical Achievement Award.

Steve Lindberg, an ESD senior research staff member, received his Ph.D. degree from Florida State University and joined ORNL in 1974. He is recipient of the Alexander von Humboldt Fellowship Award (Federal Republic of Germany) and Martin Marietta Energy Systems awards for technical achievement and publications. He is editing a book, *Sources, Deposition, and Canopy Interactions of Atmospheric Acidity,* and is chairman of the U.S. National Atmospheric Deposition Program.

Dick Olson, an ESD research staff member since 1974, has an M.S. degree in genetics from Washington State University and has become a specialist in data management and regional ecology.

Robb Turner, a biogeochemist who joined ESD in 1983 as a Wigner Fellow and is now a research staff member, has a Ph.D. degree in geology from the University of Pennsylvania.



In these ORNL field chambers, loblolly pine seedlings are exposed to acid precipitation combined with ozone. Equipped with moving, rain-activated shields, the chambers can exclude natural rain and expose the seedlings only to simulated rain of known chemistry. Loblolly pine is an important commercial timber species in the Southeast.

Global Carbon Dioxide Studies

ESD provides leadership in global carbon cycle research and information analysis.

By Michael P. Farrell

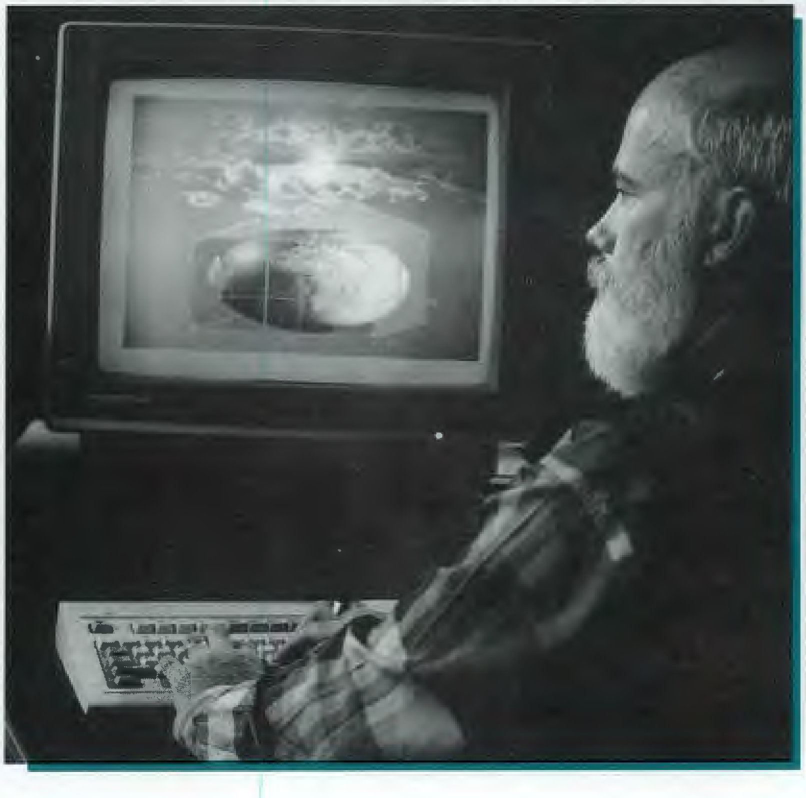
The 1988 drought renewed U.S. concern about the possibility that the Earth's climate is being altered by increasing atmospheric concentrations of trace gases, including carbon dioxide. Escalated fossil fuel combustion could increase atmospheric carbon dioxide levels enough to trap the heat radiated away by the Earth—the greenhouse effect. As a result, the temperature of the Earth's surface could rise, possibly leading to a warmer climate and shifting patterns of precipitation. Newspaper reports suggest that deserts might bloom; icebergs might melt, raising the ocean level enough to inundate coastal cities; and the "bread baskets" of today might become the "dust bowls" of tomorrow.

Whether the carbon dioxide concentrations in the atmosphere will reach devastating levels depends upon many variables, such as the amount by which fossil fuel combustion actually will increase and the extent to which the forests, oceans, and other "sinks" on the Earth can absorb the additional amount of carbon injected into the atmosphere. Thus, since the early 1970s, scientists have been studying the sources of and sinks for atmospheric CO_2—the global carbon cycle—to better understand the probability that projected industrial emissions could ultimately cause global warming.

From its early assessments of the global carbon cycle problems, the Carbon Dioxide Program at ORNL has grown substantially. Today, we are managing DOE-sponsored research in this area, including the Global Carbon Cycle Program, the Carbon Dioxide Information Analysis Center, and the Resource Analysis Program.

Global Carbon Cycle Program

The Environmental Sciences Division (ESD) has become a focal point for carbon cycle research for three reasons. First, in the 1960s, Jerry Olson in ESD was among the first to identify the importance of carbon exchange between terrestrial ecosystems (e.g., forests) and the atmosphere. As a result, ESD became one of the first groups to coordinate and support carbon cycle research. Second, ESD participated actively in the International Biological Program (IBP) by gathering, synthesizing, and distributing woodlands data and related information on the forests of the eastern



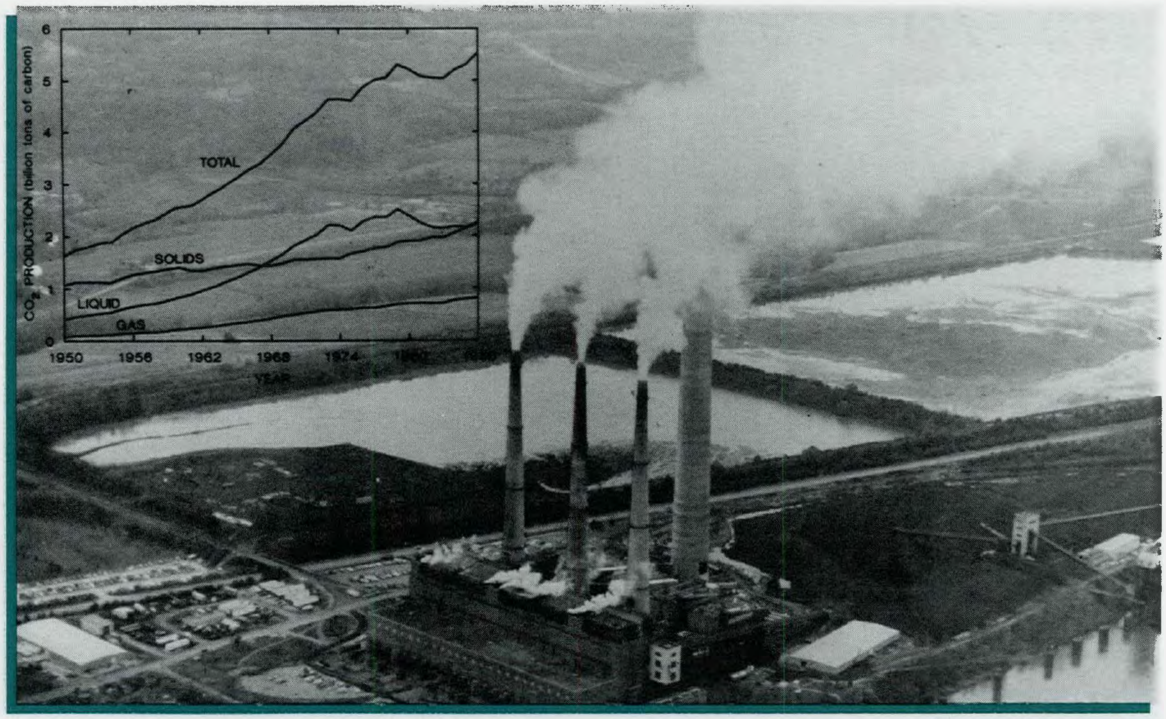
Mike Farrell directs ORNL research on the "greenhouse effect" and other possible global effects of increasing atmospheric levels of carbon dioxide. (Computer image by R. Mitchell Williamson, Energy Systems Graphics Division)

United States. Consequently, ESD established worldwide contacts for carbon data even before the Division became the recognized center for carbon cycle research management. Third, ESD's interdisciplinary staff contributes the broad range of expertise needed to perform carbon research. In particular, ESD has unique computer capabilities for modeling and information management.

An early ORNL assessment of the global CO_2 problem, published in 1976, analyzed the current state of knowledge, summarized efforts to fill information gaps, and suggested ways that the federal government could clarify the environmental and social costs or risks of climatic change. In 1978, with support from the National Science Foundation, the Global Carbon Project was developed to determine whether the combination of forest and woodlands is a net source of, or sink for, atmospheric CO_2. (Although green plants absorb CO_2, burning plant material also makes forests a source of CO_2.) Another project goal was to develop a means for predicting changes in global carbon balance.

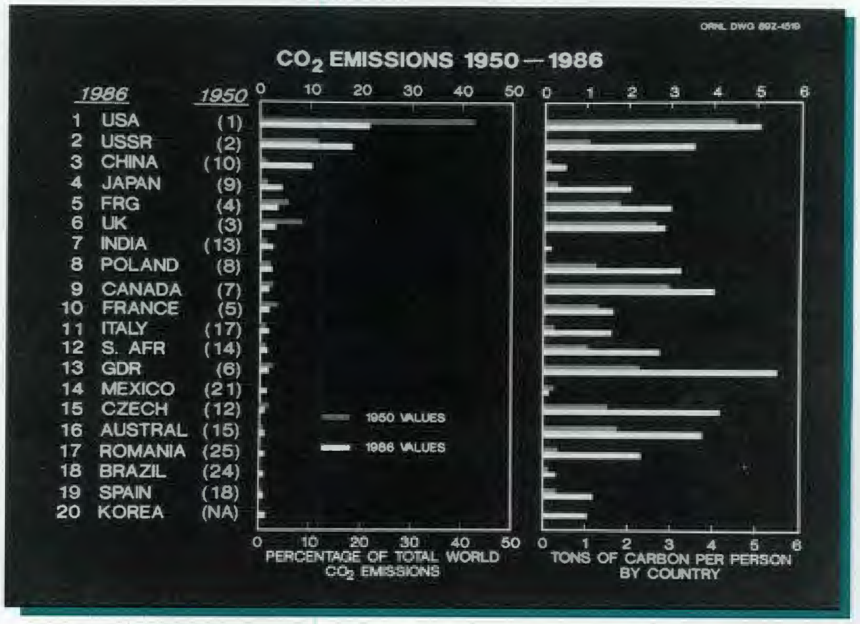
In 1982, ESD began providing managerial guidance and performing needed research for the DOE national Carbon Dioxide Research Program. Using its extensive background in carbon biogeochemistry research and computer modeling, ESD managed and participated in research to

- ☐ determine current and past atmospheric CO_2 concentrations, changes, and trends;
- ☐ estimate past and future releases of CO_2 from fossil fuel combustion;
- ☐ quantify direct effects of atmospheric CO_2 enrichment of forest tree species (see sidebar on p. 124);
- ☐ analyze the uptake of CO_2 by oceans;
- ☐ assess CO_2 exchanges between the atmosphere and the biosphere;
- ☐ evaluate additional carbon fluxes, sources, and sinks;
- ☐ develop models of the global carbon cycle; and
- ☐ project future atmospheric CO_2 concentrations.



Fossil fuel combustion to generate electricity has significantly increased the amount of carbon dioxide injected into the atmosphere.

In 1983, John Trabalka and Dave Reichle hosted the Sixth Life Sciences Symposium at ORNL entitled, "The Global Carbon Cycle: Analysis of the Natural Cycle and Implications of Anthropogenic Alterations for the Next Century." Jointly sponsored by federal agencies and industry, the symposium drew 180 scientists from ten countries. The published proceedings, which contains invited contributions by internationally recognized scientists, is still a major source of technical information on the carbon cycle.



Between 1950 and 1986, the United States and the Soviet Union were the dominant contributors to the world's carbon dioxide emissions. China and Japan now contribute a much larger share than they did in the 1950s.

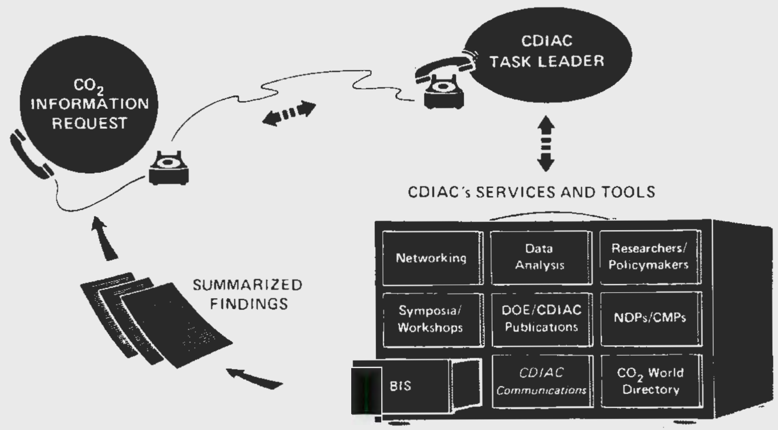
In 1985, Trabalka and other ESD scientists published *Atmospheric Carbon Dioxide and the Global Carbon Cycle*, one of DOE's major reports on CO_2 research to identify the potential environmental impacts of increased CO_2 concentrations in the atmosphere.

Since that time, the Global Carbon Cycle Program at ORNL has been realigned to conduct or oversee new research in terrestrial carbon dynamics and global carbon cycle modeling. Our management of international global carbon cycle research for DOE has expanded to focus on assembling and implementing multidimensional models of the global carbon cycle. We are also assisting in DOE's Carbon Dioxide Research Program, particularly in the collection and analysis of oceanic and atmospheric data. Our goals are to (1) develop information and models needed to provide accurate projections of CO_2 buildup in the atmosphere over the next century from both natural and anthropogenic sources and (2) evaluate the direct and indirect effects of CO_2 on forest communities through both experimental studies and mathematical simulations.

Carbon Dioxide Information Analysis Center

In 1982, the Carbon Dioxide Information Analysis Center (CDIAC) was established at ORNL to serve DOE's Carbon Dioxide Research Program. CDIAC was modeled after the information analysis center concept developed by former ORNL Director Alvin Weinberg. Serving as much more than an information clearinghouse for collecting and dispersing carbon-dioxide–related data and reports, CDIAC analyzes, evaluates, organizes, and combines much of the information before it is distributed. The Center also makes recommendations for computer hardware, software, and languages to meet the needs of researchers in the field.

CDIAC has grown from 4 staff members in 1982 to its current 12, providing information and services for over 4000 researchers and policy analysts from 147 countries. Since FY 1985, CDIAC has filled more than 11,000 requests for carbon-dioxide–related information. In early 1989 CDIAC began negotiating the possibility of becoming the tenth U.S. World Data Center. To be a World Data Center, an organization must show expertise in scientific data manipulation, have



ORNL's Carbon Dioxide Information Analysis Center offers many services and tools to meet requests for CO_2-related information about research findings on the global carbon cycle.

long-term support, and adhere to guidelines of the International Council of Scientific Unions. Becoming a World Data Center would increase our prestige in the international CO_2 research community.

One of our past projects was estimating the effects that a rise in sea level would have on coastal resources. Such effects might include destruction of coastal wetlands, inundation of structures such as roads and hazardous waste sites, and increased salinity at water intakes. These studies by Robert Cushman, Dennis Miller, and others eventually became a part of DOE's Resource Analysis Program.

Another unique project was CDIAC's involvement in a joint research effort sponsored by DOE and the People's Republic of China to study the effects of increasing atmospheric CO_2 on climate. In the initial arrangement, the United States supplied the Chinese with personal computers for the analysis and exchange of climatic and other data collected over many centuries.

Tommy Nelson of CDIAC developed and established a computer system for the project, Paul Kanciruk developed a standard operating procedure for data exchange, and we are continuing to provide technical personnel support. Over the course of the project, more than 64 million data elements will be exchanged.

The Center also distributes carbon-dioxide–related numeric data packages (NDPs) and computer model packages (CMPs) containing written documentation and magnetic tapes with data and model software supplied by contributors through cooperative agreements. The documentation includes an abstract, a data- or source-code listing (either in tabular form or on microfiche), a FORTRAN data retrieval program, graphical displays of the data or model output, and reprints of pertinent literature. Since 1985, Tom Boden and others have assembled 34 NDPs and 2 CMPs.

CDIAC also maintains a Carbon Dioxide Bibliographic Information System (BIS) data base currently containing more than 10,000 citations, organized according to the major CO_2 program areas. Searches are performed on request. The BIS serves a vital function to the research community by collecting the literature on diverse subjects related to climate change.

Resource Analysis Program

If atmospheric CO_2 levels significantly increase in the future, and if climate changes occur as a result, what will be the effects of these changes? This ultimate public concern is being addressed by ESD scientists in DOE-sponsored work, including identification of the research and data needed to resolve the major uncertainties.

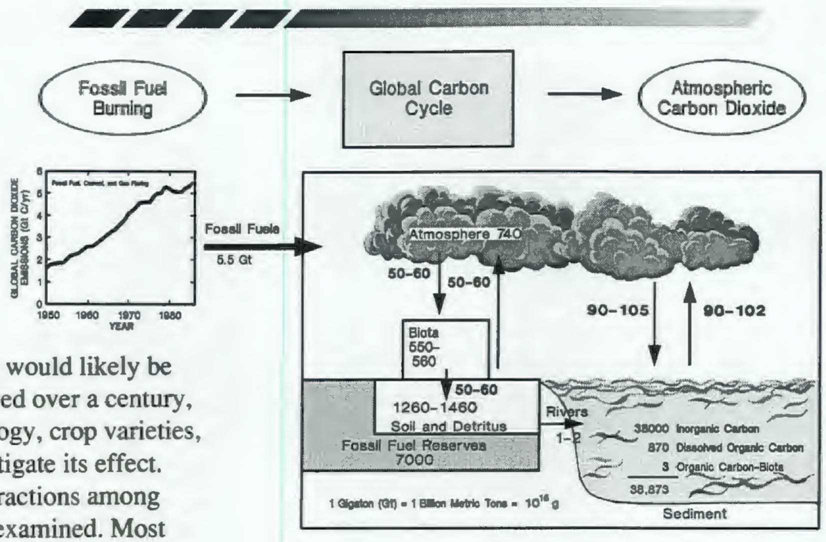
A group of ESD staff, under Bob Cushman's leadership, suggested several investigations in the area that came to be known as the Resource Analysis Program. First, the effects of both the atmospheric CO_2 itself and the carbon-dioxide–induced climate changes must be accounted for. Second, the list of resources to be considered must be expanded. Agriculture and water resources have been extensively studied in relation to the effects of climate change, but less attention has been given to fisheries, forests, and human health. Other economic and social resources known to be sensitive to weather and climate (e.g., recreation) must also be considered.

Third, the timing of the projected climate changes must be considered. These will not occur instantaneously, as some studies to date have assumed, but will evolve over decades to centuries, as will natural and societal resources. Although an immediate climate change might devastate some sectors of agriculture, the same change would likely be much less significant if it evolved over a century, because adjustments in technology, crop varieties, and farming practices could mitigate its effect.

Fourth, climate-affected interactions among resources and regions must be examined. Most studies to date have addressed single resources and generally have been limited to a single geographical area. However, important interactions occur among regions and among resources within a region. For example, agriculture and forestry compete for land use in some areas. Furthermore, agriculture in one area (e.g., Nebraska) is influenced by production elsewhere in the United States and around the world. Dave Shriner, Robin Graham, Carolyn Hunsaker, and Don DeAngelis are working to identify many of these important interactions.

Such a broad-ranging Resource Analysis Program is clearly multiyear, multidisciplinary, and multinational in dimension. Some of the important questions to be addressed are: How do we define climatic "regions"? How can the interactions among resources and regions be modeled for various spatial and temporal scales? How can society's structure and needs be predicted for the next century, when the expected climate changes may occur? How will various societal structures respond to the predicted changes in climate?

These questions go beyond the traditional scope of environmental sciences and encompass other disciplines, such as political science and economics. Thus, in Resource Analysis, ESD will be working with a larger group, including physical scientists, social scientists, economists, and engineers from throughout ORNL as the DOE program matures. ornl



Responses of atmospheric carbon dioxide concentrations to fossil fuel releases are controlled by the carbon cycle.

Michael P. Farrell is director of the Carbon Dioxide Information Analysis and Research Program of the Environmental Sciences Division. He received a Ph.D. degree in zoology and physiology from Mississippi State University and joined the ORNL staff in 1979.

CO_2 Researcher Honored

In early 1989, a carbon dioxide researcher received the annual Scientific Achievement Award of the Environmental Sciences Division. Tsung-Hung Peng was honored for "his unique creativity and perseverance in characterizing the basic processes of oceanic circulation and carbon dioxide cycling between the atmosphere and the ocean."

ESD Staff Member Testifies before Senate Committee on Reforestation

In 1988, Gregg Marland of the Environmental Sciences Division presented testimony to the Senate Committee on Energy and Natural Resources at a hearing on "The Effect of Global Climate Change on Domestic Forest Resources and the Options for Offsetting Carbon Dioxide Emissions." The hearing was prompted by a bill of the committee chairman, Senator Timothy Wirth of Colorado, entitled the National Energy Policy Act of 1988. This bill sets a national goal of achieving a 20% reduction in the 1988 level of CO_2 emissions by the year 2000.

In his testimony, Marland spoke about the potential value of planting new forests to help remove excess CO_2 from the atmosphere. Marland's estimate, reported in an October 7, 1988,"News and Comment" article in *Science*, was that 7 million square kilometers of trees—about the same area as Australia—would have to be planted to absorb 5 billion tons of carbon per year, roughly the amount of carbon released each year by the international combustion of fossil fuels. His calculations are based on the carbon-fixing abilities of the American sycamore. Marland also stated that it would be necessary to either prevent these extra trees from decaying and giving back additional CO_2 to the atmosphere or to substitute them for fossil fuel for generating power to reduce net CO_2 emissions.

Regarding climate change and domestic forest resources, Marland made three points in his testimony: (1) although carbon storage is greatest in the most mature forests, carbon uptake is greatest in young, healthy forests; (2) net annual yield per acre of reforestation must increase (i.e., through intensive management) to make it feasible for the world's forests to remove significant amounts of CO_2 from the atmosphere; and (3) the issue is truly global in nature, and domestic solutions alone cannot solve the problem.

Trees Show Complex Response to Elevated CO_2 Levels

Carbon dioxide from the atmosphere is the source of carbon for plant growth, so increases in atmospheric CO_2 concentration could influence the productivity and composition of the Earth's forests. Many studies have shown that, in an atmosphere enriched in CO_2, crop plants grow faster or larger and seed production and proportions of roots, stems, and leaves are frequently altered. Any one or a combination of these changes could affect plant succession and production in natural ecosystems. Unfortunately, trees are more difficult to study and less effort has been devoted to understanding tree responses to elevated CO_2 levels.

Because forests are responsible for about two-thirds of total global photosynthesis (conversion of atmospheric CO_2 to biomass), they are important to the global carbon cycle. Besides absorbing atmospheric CO_2, forests may release CO_2 to the atmosphere as their plant material decays or burns, constantly affecting the global carbon balance.

Scientists in the Environmental Sciences Division (ESD) are studying both natural and artificially controlled environments to try to quantify the direct effects of a CO_2-enriched atmosphere on trees and to describe the physiological mechanisms responsible for the observed effects. Rich Norby, Gerry O'Neill, and Bob Luxmoore are focusing on growth changes, belowground symbiotic microbe-tree relationships, nutrient use, and water-use efficiency in tree seedlings exposed to higher-than-ambient CO_2 levels.

Inset at top: These 12-week-old shortleaf pine seedlings grew faster than normal when exposed to higher-than-ambient concentrations of carbon dioxide and no other changes. Inset at bottom: The cores, taken from trees in this longleaf pine stand in south Georgia (main photo), show a significant increase in diameter growth beginning earlier in this century. The current ambient CO_2 level is ~350 ppm, whereas pre-industrial levels (before 1850) are estimated to have been ~270 ppm.

These researchers have found that coniferous and deciduous seedlings generally respond to elevated CO_2 levels by producing significantly more roots than normal, as well as additional aboveground biomass. The activity of symbiotic, root-associated microorganisms that help trees acquire nutrients increases when seedlings are exposed to elevated CO_2 levels. In addition, tree seedlings grown in CO_2-enriched atmospheres require less water, suggesting that such trees might be less susceptible to drought (a predicted outcome of the "greenhouse effect," theoretically caused in part by increased atmospheric CO_2 levels).

The supply of essential nutrients such as nitrogen is often limited in forests; such deficienies could influence the response of trees to increasing CO_2 levels. Experiments with tree seedlings grown in pots containing nutrient-poor forest soil have shown that nutrient deficiency does not preclude a growth response to elevated CO_2 levels. For example, in a CO_2-enriched atmosphere, seedlings grew more rapidly even though nitrogen uptake did not increase, suggesting that their nitrogen-use efficiency had improved.

What do the results of these relatively short-term experiments on tree seedlings suggest about the effects of increased atmospheric CO_2 concentrations on real world forests? So far no one knows for certain, but the growth history of trees, recorded in their annual rings, may provide part of the answer. Working with natural stands of longleaf pine in the southeastern United States, Tom Doyle (Maxima Corporation), Lynn Tharp (Computing and Telecommunications Division), John Beauchamp and Darryl Downing (Engineering Physics and Mathematics Division), and I (ESD) recently discovered an unexplained increase in tree growth that began in the 1920s. This growth rate continues to rise, and annual wood production is now about 35% greater than expected. This increased growth could be the result of a variety of environmental factors, but we have eliminated potential causes such as site manipulation and changes in precipitation, temperature, and atmospheric nitrogen and sulfur levels. If the growth rate proves to be accelerated by rising atmospheric CO_2 levels, the consequences for global forest growth and use could be important. Simultaneous with a global warming, which could greatly reduce rainfall in some regions, forests may grow at an increasing rate despite drought conditions.

Current and future work in ESD will be directed toward obtaining data pertinent to understanding longer-term responses of trees to CO_2-enriched atmospheres and the ecological consequences to the Earth's forests.—*Darrell C. West, Environmental Sciences Division.*

Producing Energy Crops

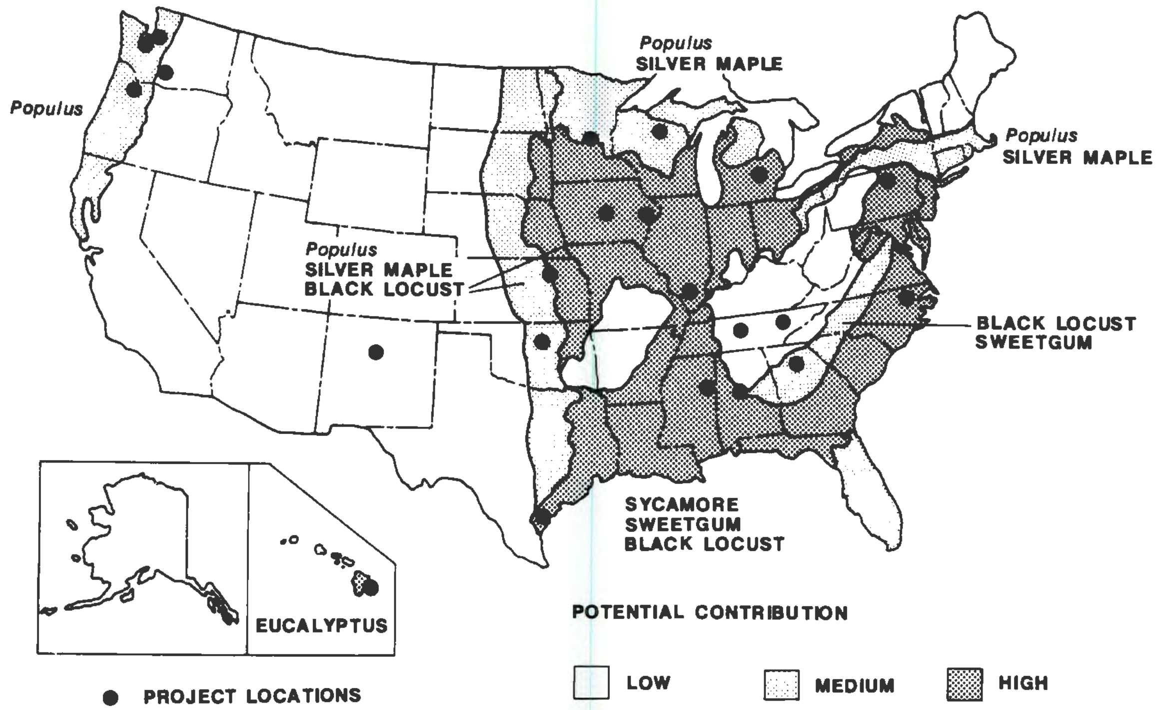
By J. W. Ranney, J. H. Cushman, P. A. Layton, L. L. Wright, and A. F. Turhollow

ORNL is spearheading a research effort that could help farmers use their land more efficiently and provide a significant domestic energy source for the nation. The project focuses on biomass—organic matter derived from trees, shrubs, grasses, manure, and municipal waste. Biomass production can be both an alternative for farmers facing shrinking markets for conventional crops and an alternative to petroleum as an energy source. In fact, biomass is the only domestic source of renewable energy that can provide a liquid transportation fuel. Biomass could supply about as much liquid fuel as U.S. petroleum imports (about 20 EJ/year of alcohol, gasoline, and diesel fuel). It could help make the United States energy self-sufficient. Public concern about fossil carbon accumulation in the atmosphere and the prospect of alternative farm crops have also increased interest in biomass crops.

Pat Layton measures the diameter of an American sycamore on the Oak Ridge Reservation to help estimate its biomass.

The Department of Energy's research on biomass energy systems covers both growing biomass and converting it to biofuels. ORNL provides leadership in the research related to growing new herbaceous and wood energy crops, managing programs that now involve more than 25 research institutions. Part of our responsibility is to ensure that the energy crops are appropriate for the thermochemical and biochemical conversion systems under development by other DOE laboratories. Together with these laboratories, we are working on biomass analysis and testing and on technical and economic analyses of biomass energy systems.

The goal of DOE's biomass production research is to develop new energy crops that maximize energy production while minimizing cost. Research has focused primarily on very-fast-growing trees and grasses. Such crops can be grown inexpensively and the entire aboveground production becomes the energy feedstock, rather than just a portion of it, as in conventional food or fiber crops. Secondary emphasis is on oil production from



This map shows the location of short-rotation woody crop research throughout the United States and indicates the potential for energy crops in these areas.

winter rapeseed and microalgae. Research to date has addressed several major areas for improving terrestrial (land-based) crops to meet large energy demands and specific energy requirements. Some of the research activities are (1) screening native and exotic species for fast growth and other capabilities, (2) testing innovative cultural systems, (3) identifying physiological and chemical characteristics important for crop improvement, (4) breeding improved species, and (5) coordinating biomass handling and economic evaluations. This U.S. program is leading the world in physiologically defining plant growth mechanisms for genetic improvement.

Short-Rotation Woody Crops

Short-rotation woody crops are closely spaced, fast-growing trees raised in plantations under intensive culture (similar to that used for agricultural crops) and harvested every 3 to 10 years. The concept is appropriate for marginal-to-good agricultural land and can achieve biomass productivity rates of up to 12 to 20 Mg/ha/year, much higher than eastern Tennessee's natural-stand productivity of about 2 to 4 Mg/ha/year.

Over 140 tree species, mostly hardwoods and a few pines, have been evaluated for fast-growth capability in hundreds of experiments throughout the United States. In many regions, 20-cm-diam (8-in.) full-sized trees can now be grown and harvested in 5 to 8 years. Growth is a good predictor of energy production. Hardwoods have been given the most attention because some show faster early growth than pines, and they generally have the ability to resprout from stumps once they have been cut, saving replanting costs. Hardwoods also respond much better to intensive silvicultural practices. Research has defined the species and site limitations for wood energy crops and has helped to develop successful establishment and crop-tending techniques.

The rapid-growth characteristics of the genus *Populus* (poplar, cottonwood, aspen), as well as four other species (silver maple, American sycamore, black locust, and sweetgum), are being evaluated to identify the physiological processes responsible for their superior growth and to learn how they can be further improved. Because of their superior growth, they have been identified as model species on which to focus research. Limited research in the semiarid Southwest has identified mesquite and fourwing saltbush as species of interest. The map on the previous page shows sites of biomass experiments and suggests the large-scale potential for wood energy crops in these areas.

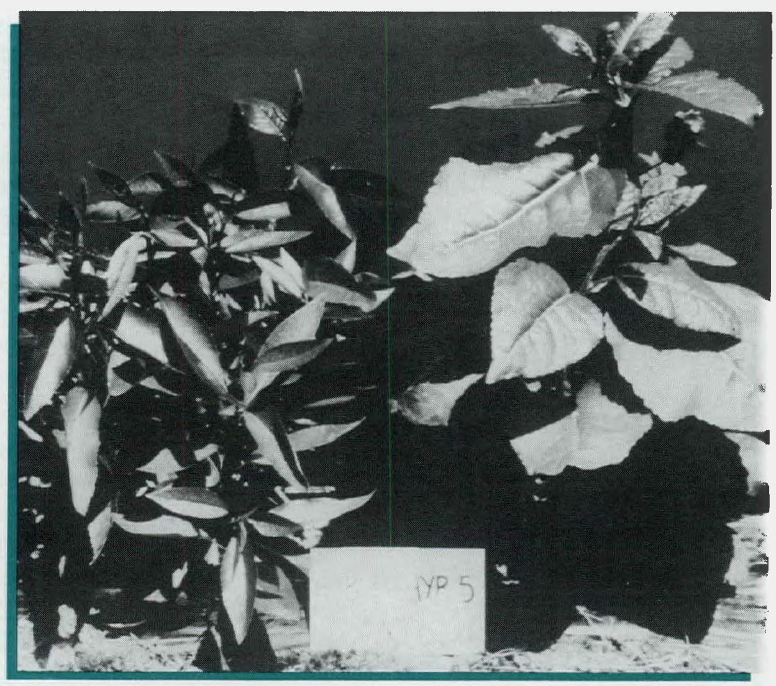
Growth at research sites has been extremely variable, and in several cases, surprisingly high (see table at end of article). What has been even more surprising is that growth in large-scale commercial trials has been only about 10% less than growth in experimental plantings, whereas 50% shortfalls were anticipated. A data base on short-rotation intensive culture documenting the experimental results of DOE-funded research has been developed at ORNL. Analysis of the data base information can assist in understanding the factors affecting growth responses on a continental scale.

The short harvest cycles and high productivity characteristics of short-rotation forestry provide an economic opportunity to small landowners. Intensive cultural practices such as weed control, fertilizers, and pest/disease control can maximize growth capacity. Genetic studies have also identified tree gene pools that can be used to further improve growth stocks.

In *Populus*, important traits contributing to high productivity in short-rotation systems include narrow crowns with small limbs, larger upper leaves and smaller lower leaves, indeterminate growth habit (the stem elongates throughout the growing season), pest resistance, and drought tolerance. Other traits currently under investigation at ORNL and elsewhere are root dynamics, leaf display for maximum light interception, photosynthesis, and dry-matter partitioning. As we learn more about genetic variation, growth, culture, and conversion technologies, additional desirable traits can be defined and selected, bred, or genetically engineered to produce superior growth stock.

Physiological investigations have detailed some of the reasons for the vigor observed in new poplar hybrids. Black cottonwood, with large leaf cells, and eastern cottonwood, with many leaf cells, combine to produce hybrids having many large leaf cells, which result in bigger leaves and faster tree growth (see photo below). Arrangements of stomata affecting leaf gas exchange, leaf morphology, and disease resistance may also contribute to the hybrid's vigor.

Other species are being improved in an effort to expand their ranges to poorer quality sites and more regions, such as the South and the Great Plains. In addition to genetic screening and physiological studies, efforts are being made to develop propagation methods (including tissue culture) for clonal research and regeneration of a large number of plantlets. Somaclonal screening and recombinant DNA research are already being



The variability of hybrid cottonwoods is illustrated by the difference in leaf size.

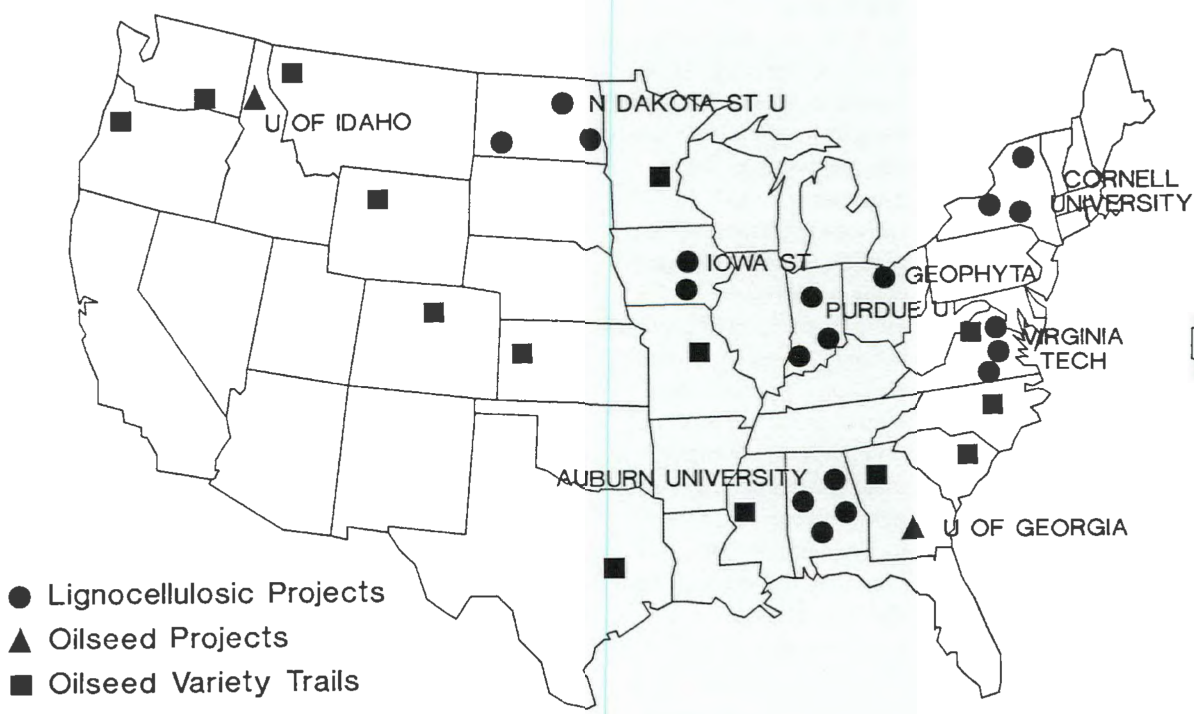
applied to several tree species to improve traits such as disease resistance or herbicide tolerance.

These techniques will also be used to develop species with chemical qualities helpful to biofuel conversion—which cannot be done through classical breeding approaches. Moreover, biotechnology offers tremendous potential for advancing our understanding of biochemical pathways in plants that may serve many other needs.

Research on woody species indicates that maximum productivity can be increased at least another 50 to 100% over current levels through genetic improvement. Genetic research and breeding should reduce silvicultural costs to less than $2/GJ (about $34/dry ton) by developing crops that are more easily tended and have greater potential growth. In addition, the chemical composition of trees can be manipulated to improve biofuel conversion efficiency by at least 10%.

Herbaceous Crops

The screening of grasses and other herbaceous lignocellulosic crop species, the herbaceous equivalent of wood energy crops, is being conducted in the Southeast, Midwest/Lake, and Plains states (see map below). These five-year projects, now in their first through fourth years, have some common features. Each is designed to provide the data necessary to choose the best energy-crop species for specific regions through field trials of annual and perennial grasses and legumes using a range of management regions and sites. In each region, some field-trial sites are located on prime farmland having few restrictions on crop production, whereas others are located on marginal farmland having varying constraints, such as less than ideal wetness, fertility, or erosiveness.



Locations of 20 screening-trial sites for 7 projects dealing with herbaceous lignocellulosic crops.

"The United States may soon lead the way in producing new biomass energy crops."

Yields of both annual and perennial species have been impressive. Annual sweet sorghum has produced >25 dry Mg/ha in both the Southeast and Midwest/Lake states. Another 4 to 5 Mg/ha of annual rye has been grown as a winter crop on the same sites for a biomass total annual productivity of 29 to 30 Mg/ha. Perennial switch-grass has produced over 12 dry Mg/ha under dry conditions in Indiana and over 9 dry Mg/ha in Virginia during the second year of a record-breaking drought. These studies demonstrate the productive potential of herbaceous crops.

An additional experiment has been established at the Oak Ridge National Environmental Research Park to investigate the possibility of using successional "weedy" vegetation from abandoned fields and pastures as biomass energy feedstocks. This approach should avoid some of the costs and risks associated with establishing and maintaining monocultures of other crops. Tests in 1986 and 1987 have shown productivity comparable to newly planted stands of perennial species in Virginia and Alabama. Production in both years was influenced by unusually severe drought conditions. Production on an old pasture ranged from 2.2 Mg/ha in untreated control plots to 6.8 Mg/ha in plots treated with lime and fertilizer. Production on an abandoned soybean field ranged from 4.2 Mg/ha without soil amendments to 6.6 Mg/ha with phosphorus and nitrogen applications. Harvesting and fertilizer treatments also influenced the types of plants growing on the sites.

Both annual and perennial species will probably have a place in biomass energy systems. Annual species such as sweet sorghum can outproduce perennial species when moisture is not limiting, and they respond well to high levels of nitrogen fertilizer. However, annual species are more severely affected by drought. In years of adequate rain and normal temperatures, production costs have been much lower for annual sorghums than for perennial grasses. The opposite has been seen in drought years. These observations suggest that the sorghums are high-input, high-output, and relatively high-risk systems, and the perennial species are low-input, moderate-output, and relatively low-risk systems. Environmental and farm-system considerations also differ. Without the use of winter cover crops, annual species leave the soil exposed to erosion during late fall, winter, and early spring. Perennial species are much more appropriate for erosive soils. Timing of harvest is more critical for sorghums than for most perennial crops, and overall production costs may be higher because the shorter harvesting period may lead to less efficient machinery use.

After the screening phase of the herbaceous crop research is completed, breeding programs similar to those described for woody crops will be started for the most promising species. The transition from screening to breeding will begin in 1989.

Conclusion

A wide range of sites in the United States can be used if both herbaceous and woody crops are cultivated for the production of biomass as a renewable energy feedstock. Current results from advanced breeding methods and biotechnology applications indicate that we are in the very early stages of improving productivity and that advances can be made rapidly. We have already identified species showing great promise (see table on next page). Improving productivity, combined with increasing the variety of sites on which these new crops can grow, creates new opportunities for the United States to become energy self-sufficient. To attain the 10 to 20 EJ/year of biomass energy that is believed possible requires continued cultural and genetic research in drought tolerance, nutrient-use efficiency, and the allocation of growth to harvestable parts of the plants. No one system suits all situations; different crops and cultural systems are needed to achieve the best economic and production results over the range of sites and conditions.

Dedicated energy crops offer the American farmer a valuable alternative at a time when many conventional crop markets are shrinking. Using available technology, prime-to-marginal row-crop land can be used to produce 20 dry Mg/ha/year or more of energy crops in the near future. The United States may soon lead the way in producing new biomass energy crops. ornl

Productivity of U.S. Short-Rotation Wood Energy Plantations

Species, site	Biomass[a] dry Mg/ha/year (dry tons/acre/year)	Harvest age (years)
Eucalyptus, Florida, Hawaii	12–27 (5–12)	3–6
Populus sp., northern United States	12–43 (5–20)	1–8
Silver maple, Midwest and Great Plains	12–18 (5–8)	5–8
American sycamore, Southeast	12–18 (5–8)	4–5
Black locust, Midwest, Great Plains, and South	12–18 (5–8)	4–8
Sweetgum, Southeast	12–16 (5–7)	5–12

[a]Productivity of <12 dry Mg/ha/year (5 tons/acre/year) currently is not considered potentially successful for energy plantations. Future definition of energy qualities and physiological characteristics could cause these data to change. Lower-productivity species have not received as much research and development attention.

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Janet H. Cushman, who joined ESD in 1974, holds an M.S. degree in ecology and evolution from Yale University. She is acting program manager for ORNL's Biomass Production Program and field manager for the Herbaceous Energy Crops Program.

Patricia A. Layton joined the ESD staff in 1985 after receiving her Ph.D. degree from the University of Florida. She is task manager for genetics, physiology, and biotechnology in the Short Rotation Woody Crops Program.

Lynn L. Wright of ESD is acting field manager of the Short Rotation Woody Crops Program. She received an M.S. degree from Ohio State University and began work at ORNL in 1974.

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Environmental Assessment

By Stephen G. Hildebrand

A major mission of ORNL's Environmental Sciences Division (ESD) is to develop and apply methods and analytical tools for both quantitative and qualitative assessment of environmental issues. The theme of environmental assessment permeates most of the division's activities. Field and laboratory research projects are often justified based on their ability to provide information needed for environmental assessments. These, in turn, often identify research needs when the consequence of particular actions cannot be predicted with a satisfactory degree of certainty. This feedback loop between assessments and research is important to ESD's vitality.

"When the EPA needed to apply the concept of risk assessment to the environment, they turned to ORNL."

NEPA Process

Environmental assessment in ESD takes many forms. Our assessments in support of the National Environmental Policy Act (NEPA) in the early 1970s gave us an entry into this field. Almost all ESD staff members in the early 1970s participated in some way in the NEPA process and have many exciting stories to tell.

During the 1970s, participation in the NEPA process for nuclear power plants often entailed testifying at formalized hearings. The scrutiny given to environmental analyses in these hearings often was more extensive than that received from peer-reviewed journals. Cross-examination by an attorney scrutinizing our scientific work was a new, and often traumatic, experience for all. Through the 1970s and into the 1980s, technical analyses in support of NEPA continued. In partnership with the Energy Division and the Health and Safety Research Division, ESD ecologists analyzed the environmental impacts of (1) constructing and operating nuclear power plants; (2) constructing and operating synthetic fuels plants; (3) developing geothermal energy; (4) mining, milling, and fuel fabrication for the nuclear industry; (5) producing alcohol fuels; and (6) developing hydroelectric resources.

Recently, the environmental impacts of major projects sponsored by the Department of Defense have been addressed. The interdisciplinary teams that ORNL can muster in support of NEPA analyses are unequaled. A companion article by Bob Reed and his colleagues (see p. 134) chronicles our NEPA expertise in more detail. We are proud of our accomplishments and look forward to continued involvement in this area.

In 1984, William Ruckleshaus, administrator of the Environmental Protection Agency (EPA), advocated the use of risk assessment in environmental regulation. ORNL already had extensive experience applying risk analysis to reactor operations, primarily from an engineering perspective, and was involved in developing an understanding of the human health risks associated with synthetic fuel plants and nuclear power stations.

When the EPA needed to apply the concept of risk assessment to the environment, they turned to ORNL. Larry Barnthouse, Glenn Suter, Bob O'Neill, Bob Gardner, and Steve Bartell pioneered the development of ecological risk assessment methods for EPA, starting with several lively brainstorming sessions that included ESD staff from both traditional research and environmental assessment areas. What evolved was an approach for predicting the probability of adverse environmental effects based on our research in ecosystem modeling and environmental toxicology. We have developed and demonstrated risk protocols for the effects of toxic chemicals and have used these for assessments of toxic chemical effects and other stresses at ever-larger spatial scales, up to and including regional concerns such as acidic deposition and other air pollution effects.

Regulatory Compliance

Environmental assessments resulting from federal regulatory programs constitute the newest challenge for ESD and other divisions at the Laboratory. Drawing on our NEPA experience and on other technical expertise in the division and the

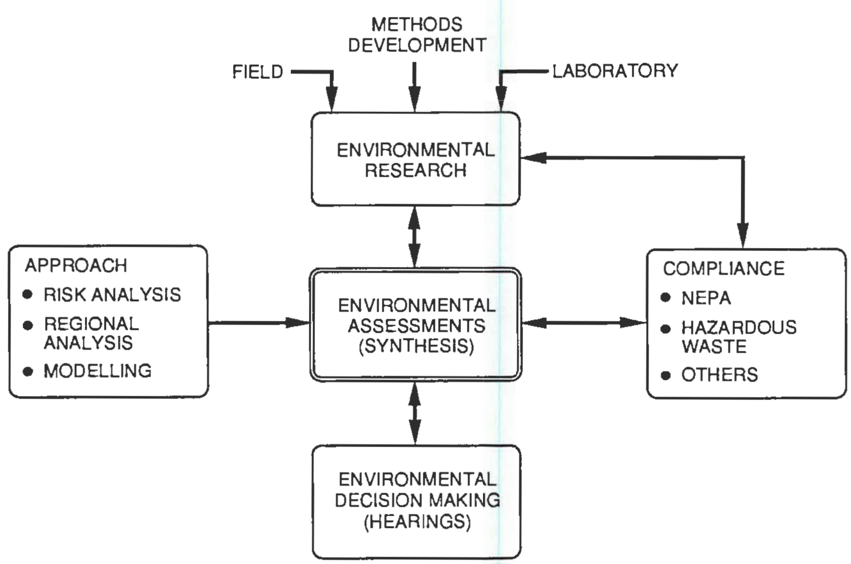
Laboratory, we are providing guidance to the Department of Energy to ensure compliance of their facilities with major regulatory programs. This is a tremendous challenge because the regulatory requirements are extremely complex.

By assisting DOE and the Oak Ridge facilities in understanding and implementing compliance with air, water, and waste regulations, the ESD staff has become directly involved both in field studies for collecting environmental information and in the follow-up laboratory work and assessment preparation. ESD regulatory specialists target the environmental information needed, and research staff collect the needed data to characterize specific environments of the Oak Ridge Reservation. These focused research and assessment programs directed at achieving environmental compliance are expected to be models for other DOE facilities.

The overriding focus of our environmental assessment activities is on providing technical information and insights that are useful for decision makers. Although the ultimate decisions might not always be dominated by environmental concerns, our continuing challenge is to make sure that the environmental issues are clearly articulated, the technical aspects of the issues are understood by the decision maker, and the environmental consequences of the proposed actions are quantified to the maximum extent possible. ornl

Steve Hildebrand came to ORNL in 1973 after receiving his Ph.D. degree in fisheries from the University of Michigan. He heads the Environmental Analyses Section in the Environmental Sciences Division, teaches a course in the University of Tennessee's Graduate Program in Ecology, and works with the UNESCO International Hydrological Programme investigating the relationship between energy policies and water resources.

"The overriding focus of our environmental assessment activities is on providing technical information and insights that are useful for decision makers."



Interactions among research, assessment, and decisions

Ecology and NEPA: 20 Years and Still a Challenge

By Robert M. Reed, Michael J. Sale, and J. Warren Webb

In late 1969, Congress formally recognized a growing national concern about the environmental impacts of major federal projects by passing the National Environmental Policy Act (NEPA), Public Law 91-190. NEPA became the cornerstone of the federal government's environmental protection program, which was subsequently expanded to include laws promoting clean air and water, control of hazardous and toxic compounds, and protection of endangered and threatened species.

NEPA specifically requires all federal agencies to consider the environmental impacts of proposed projects by preparing an environmental impact statement (EIS) for any major action that might significantly affect the quality of the human environment. Compliance with NEPA involves a major commitment of time and money for many federal agencies; in 1985, for example, federal agencies published 549 EISs, many of which required one to several years to prepare.

ORNL's Environmental Sciences Division (ESD) has worked in partnership with the Energy Division in helping a number of federal agencies comply with NEPA over the past 20 years. The research interests of ESD staff researchers in the movement of radionuclides through various compartments of the environment and in biogeochemical cycling of nutrients and pollutants through watersheds and aquatic ecosystems naturally led to research that evaluated effects of human disturbances to ecosystem function and structure.

Bob Reed (right) and his colleagues (from left) Warren Webb, Mike Sale, and Steve Railsback discuss the results of ORNL's environmental impact analyses of small hydroelectric developments conducted for the Federal Energy Regulatory Commission.

Early Activities for NRC

In late 1971, shortly after the courts ruled that EISs should be prepared by a third party to ensure objectivity in evaluating environmental impacts, the Nuclear Regulatory Commission (at that time part of the Atomic Energy Commission) asked ORNL to assist in preparing independent environmental analyses for licensing nuclear power plants. ORNL staff involved in these early studies had a major influence in defining the types of analyses needed and in developing assessment approaches that could be defended both technically and legally. Initially, ESD played the lead role at ORNL in preparing EISs on nuclear power plants, but as the Energy Division assumed management of these projects, ESD's efforts focused on the analysis of ecological issues, such as (1) entrainment and impingement of aquatic organisms in power plant cooling systems, (2) effects of heated and chlorinated effluent on exposed aquatic systems, (3) impacts of cooling tower drift on surrounding terrestrial and aquatic ecosystems, and (4) disturbance to wildlife and vegetation from construction of transmission lines.

ESD's involvement in evaluating impacts of nuclear power plants along the Hudson River led to new research in the mid-1970s. Over a ten-year period as part of the Hudson River Power Project, ORNL researchers supported the Environmental Protection Agency (EPA) by developing models to predict the effects of entrainment and impingement on fish from cooling water intakes and to determine the thermal effects of cooling water from power plants on aquatic organisms.

A Shift in Priorities

In the late 1970s and early 1980s, increased emphasis on the development of alternative energy sources resulted in a shift away from assessing the impacts of nuclear power plants toward evaluating the impacts of new, unproven energy technologies. A major challenge of these projects was in evaluating the impacts of emissions and effluents from technologies with which there was no previous experience. During this period, ORNL staff prepared NEPA documents for six major DOE synfuel demonstration projects, numerous geothermal developments, and a number of alcohol fuel plants.

During this same period, the President's Council on Environmental Quality issued new procedural regulations for complying with NEPA. These new rules required agencies to include a detailed analysis of alternative actions and a public scoping process to identify significant environmental issues in any new project. ORNL staff assisted sponsors in developing some of the first EISs under these new procedures, thus helping define a now standard approach to conducting NEPA reviews and analyses.

Regional and National Projects

In recent years, ORNL staff have prepared NEPA documents for a variety of projects that have potential environmental impacts of national or regional importance. These activities have involved us in such projects as preparing (1) an EIS for the Army on its program for disposing of lethal chemical agents and munitions at storage areas throughout the country, (2) an environmental assessment for EPA on the impacts of sampling lakes in wilderness areas in the western United States as part of that agency's national assessment of acidic deposition, and (3) a series of EISs and special studies for the Federal Energy Regulatory Commission (FERC) on hydropower development in major river basins and nationwide. Our efforts for the FERC include projects on both a regional and national scale.

Since 1983, ORNL staff have prepared NEPA assessments on a number of proposed hydropower projects for the FERC. ESD staff analyzed impacts on terrestrial and aquatic resources in conjunction with Energy Division staff members, who analyzed effects on other resources, including recreation, aesthetics, and socioeconomics.

Our initial task for the FERC was a joint effort with Argonne National Laboratory preparing a draft environmental impact statement (DEIS) on the Alaskan Susitna Hydroelectric Project. A two-dam, 1620-MW hydropower development was prepared to meet the energy needs of the Railbelt of south-central Alaska (Anchorage, Fairbanks, and environs). The project was to include the highest dam in North America and would have been the largest and most expensive nonfederal hydroelectric project ever licensed by FERC.

ESD staff evaluated the effects that altered flow regimes from the dams might have on the salmon fishery of the Susitna Basin and Cook Inlet. Using the very limited information available on the effects of hydroelectric development in subarctic regions and on rivers the size of the Susitna River, we found that regulated flows from the project would significantly reduce the water surface area of side sloughs and increase their isolation from the main channel. Because these are the most critical areas for salmon spawning, we recommended mitigation in the form of postproject habitat improvement and/or supplemental stocking of three salmon species.

Publication of the DEIS generated extensive public controversy. Eventually the license application was withdrawn because of the controversy surrounding the project and an inability to demonstrate a real need for the power.

Another study, of hydroelectric development in the Owens River Basin in California, provided an opportunity for ORNL staff to develop one of the first cumulative impact analyses of a river basin for FERC. Among the ecological issues we analyzed was the impact of hydropower development on riparian vegetation (vegetation growing close to streams and rivers that is dependent on the higher water table). In the arid climate of the eastern Sierra Nevada, the riparian zone of mountain streams is a limited and valuable resource, accounting for only 1% of natural habitat in the Owens Valley. Dependent on snowmelt from upper elevations for survival, an estimated 25% of the resource has already been lost as a result of stream diversion. Our assessment of the potential impacts of seven proposed hydropower projects in

Mike Sale and Kris Dearstone use ESD's Geographic Information System to locate and analyze the impacts of proposed hydroelectric development in the upper Ohio River basin.

the basin emphasized possible long-term effects, such as loss of riparian area and changes in canopy structure (e.g., from tall pines to shrubby willows).

We found that four of the seven proposed hydropower projects had potentially serious effects on riparian plants because of partial dewatering of stream channels. Such losses would, consequently, affect terrestrial and aquatic biota, recreation, aesthetics, and (via these effects) the local economy. The cumulative, potential long-term losses were estimated to be 5% of the region's remaining riparian resource, in addition to the 25% already lost. FERC adopted the major recommendations of this EIS, denying licenses to most applicants and requiring extensive mitigation measures for the other projects.

For an EIS published by FERC in September 1988, ORNL evaluated the environmental impacts of 24 proposed hydroelectric projects at 19 existing dam sites. Hydroelectric development in the Ohio River basin consists primarily of retrofitting existing locks and dams with new turbines and generators. The proposed projects would create a new generating potential of 684 MW. Construction and operation of these facilities could have significant cumulative environmental impacts on dissolved oxygen, recreational fishing, and river navigation.

Our analysis included extensive hydraulic and water quality modeling of approximately 500 miles of river in the upper Ohio River basin. We also used a systematic, river-basin–scale perspective to examine tradeoffs between hydropower development and environmental quality and provide consistent information for licensing decisions (e.g., identifying the sites that should be licensed and the operational constraints that should be placed on them). Our assessment recommended that four of the sites not be developed. In addition, we recommended minimum spillage flows and other mitigation measures for each of the remaining projects. This NEPA document is one of the first to develop a truly quantitative approach for evaluating cumulative impacts.

On a national scale, we have recently prepared a study for FERC under the Electric Consumers Protection Act (ECPA). In passing this act, the Congress required FERC to conduct a broad study on the impacts of this legislation, which included many revisions to the federal laws regulating hydroelectric projects, strengthened the mandate for environmental protection, and rescinded developmental incentives for small-scale hydroelectric development at sites that require construction of new dams or diversions. Our work investigated the elimination of financial incentives to develop hydropower sites requiring new dams or diversions. Our evaluations included water quality, water use, fisheries and other aquatic biota, recreational opportunities, and impacts to wetlands.

Future Directions

Our NEPA work continues to challenge the ESD staff. Many of our current projects are exceedingly complex and often have high public visibility. To maintain our ability to provide state-of-the-art assessments, we must increase our use of quantitative assessment techniques and develop innovative ways of dealing with such problems as assessing cumulative impacts. Our interactions with other ESD researchers involved in risk analysis, environmental systems analysis, landscape ecology, and global climate change help us prepare high-quality environmental assessments. ornl

Robert M. Reed joined ESD's Environmental Impact Program in 1977 and is now leader of the Environmental Assessment Group. He received a Ph.D. degree in plant ecology from Washington State University.

Michael J. Sale came to ESD's Environmental Impact Program in 1980 after receiving a Ph.D. degree in environmental sciences from the University of Illinois. He is leader of the Environmental Systems Analysis Group.

J. Warren Webb received his Ph.D. degree in insect ecology from Rhodes University in South Africa and joined ESD's Environmental Impact Program in 1978. He is a research staff member in the Environmental Assessment Group.

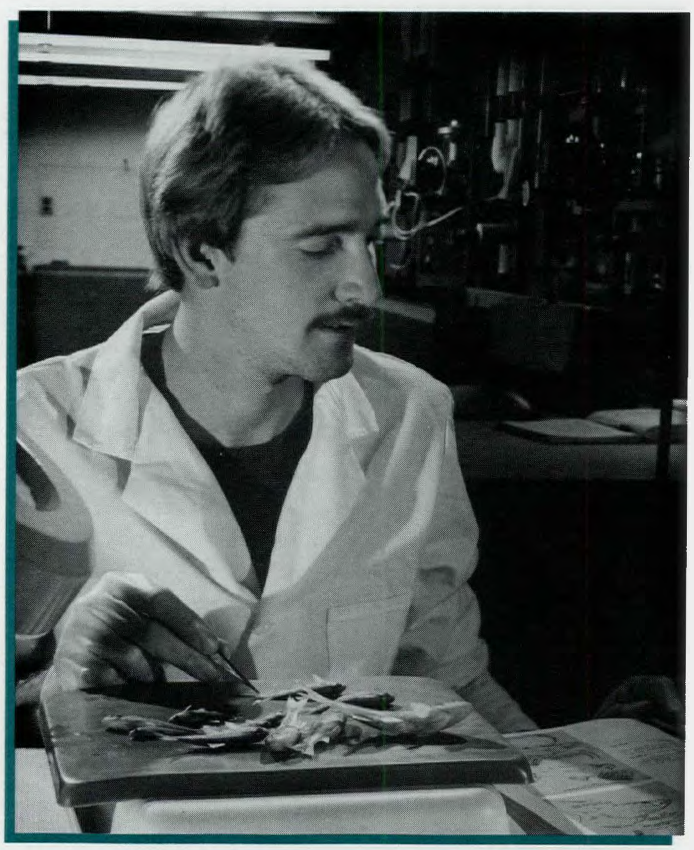
Ecological Risk Analysis

By Glenn W. Suter II, Lawrence W. Barnthouse, and Carolyn T. Hunsaker

During the early and mid-1970s, when most of the existing environmental protection laws were first enacted, the objective of most regulatory policy was to eliminate all environmental hazards or at least to reduce them to the lowest levels achievable. By the early 1980s, it became apparent that the goal of eliminating all risks could never be achieved. The current regulatory philosophy stresses balancing degrees of risk against the costs of risk reduction. Other factors, such as competing risks and risks that are generally accepted by society, must also be considered.

In 1981, ORNL's Environmental Sciences Division became involved in risk analysis We were asked by the Environmental Protection Agency (EPA) to develop methods for applying the concept of risk to regulatory problems related to the ecological effects of synthetic fuels (synfuels) and other toxic chemicals. At that time, no such methods existed, and many scientists were skeptical that they could be developed at all. Applying the concept of risk to the environment requires (1) identifying the kinds of adverse effects to be minimized, (2) estimating the magnitudes of those effects, and (3) becoming aware of the related uncertainties. All of these problems appeared insuperable.

When assessing human health risks, the ultimate effects (endpoints) are known—disease, injury, and death. In ecological risk assessment, there are no exact equivalents. The environment of a typical industrial facility is likely to support hundreds or thousands of different kinds of plants and animals, even in a desert. Because we cannot protect every organism from harm, we must determine which ones have the highest priority. Should our risk assessment be based on the percentage of all species that might be affected, or on the potential effects on a small number of "representative" species? Perhaps instead of looking at species, we should use some higher measure of "ecosystem integrity" (a legislative and regulatory term that has no clear definition and that has spawned many environmental indices).



Body characteristics of fish samples are evaluated by Mark Harris to help determine water quality of Oak Ridge Reservation streams.

Even if certain endpoints can be defined, the scientific problems that must be addressed in ecological risk assessment are complex and difficult. Direct information concerning the effects of toxic contaminants on the environment, comparable to human epidemiological data, is rarely available. It is inevitably necessary to extrapolate risk estimates from laboratory toxicity data or from limited field experiments. When we began our work, no research methods existed for making these extrapolations.

Uncertainties and Endpoints

Many uncertainties are involved in the extrapolation process, such as differences in the ways organisms are exposed to toxic chemicals in laboratory tests and in nature, variations in the sensitivities of test organisms and the organisms of interest in the environment, and the inherent variability of the environment itself. Although these uncertainties are fairly easy to identify, little was known about their magnitudes before our work, and few attempts had been made to incorporate them in assessments.

With our colleagues Bob O'Neill, Bob Gardner, and Steve Bartell, we began attacking these problems in 1981. Rather than attempting to list all possible endpoints for ecological risk assessment, we defined some general criteria for assessing endpoints appropriate to any given problem. A useful endpoint should (1) have biological relevance, (2) be of importance to society, (3) have an unambiguous operational definition, and (4) be accessible to prediction and measurement. Societal importance is a necessary factor because regulatory experience has shown that risks to insects, zooplankton, or other organisms not perceived by society as being valuable are not likely to influence decision making—unless effects on these organisms can be clearly shown to indicate risks to the fish, wildlife, crops, or forest trees that society does value. We initially identified five endpoints that meet these criteria: reductions in fish populations, blooms of noxious algae, reduced agricultural productivity, undesirable changes in forest composition, and reductions in wildlife populations.

Risk Analysis Development

To begin developing our risk analysis for toxic chemicals derived from synthetic fuels, we emphasized risks to fish populations resulting from wastewater discharges and product spills. From standardized data bases of several federal agencies, we had considerable data on the toxicity of synfuel-related chemicals to aquatic biota, particularly the effects of acute exposures on fish. A broad base of ecological theory was also available on the dynamics of fish populations and the structure and function of aquatic ecosystems (much of the latter developed at ORNL).

Using these materials, we developed quantitative methods for estimating the risks of toxic chemicals to the fish populations inhabiting typical rivers and lakes in North America. Our risk models explicitly account for the effects of test conditions (e.g., extrapolation from short-term laboratory tests to long-term exposures in the environment) and interspecies variability in sensitivity. Our methods quantify the degree to which the predicted losses of individual fish will affect the overall population. Through the use of ecosystem simulation models, they also account for contaminant-induced changes in the ability of ecosystems to support fish populations.

In our initial applications of these risk assessment procedures to synfuels, we showed that the greatest risks to nonhuman biota from synfuels were from their associated conventional pollutants (ammonia, phenolic compounds, and heavy metals), rather than the exotic polycyclic and heterocyclic organic compounds that pose the greatest human health risks. We applied these concepts to develop a system for assigning priorities to the monitoring activities at synfuels plants and for ranking hazardous waste sites for study and cleanup.

It was immediately apparent that our methods and results were widely applicable to problems involving toxic chemicals in the environment. Our synfuel work had shown, much to the surprise of many toxicologists, that the responses of various fish species to exposures from a particular contaminant are highly correlated and predictable (with varying degrees of uncertainty) using our models. We derived ways of integrating toxicity test data with existing population and ecosystem models, so that risk assessments can be based on the best available data from fisheries management and aquatic ecology. Both the EPA and the National Oceanic and Atmospheric Administration have funded additional development of our risk assessment methods at ORNL, and the Society for Environmental Toxicology and Chemistry has issued a position paper on ecological risk assessment that relies heavily on our approach.

Most of our methods are directed toward problems in which the contaminant(s) of interest have not yet been released, and for which the impacts can be addressed on a local scale. We are now expanding our approach to include risk assessments based on the observed burdens of pollutants or markers of pollutant exposure in nature. To do so, we must project forward in time to estimate implications of the exposure for the future of the ecosystem and its component populations. We must also project backward in time to determine the pollution sources and pinpoint the necessary reduction in pollutant releases.

Other problems we are now addressing involve estimations of regional-scale risks from local-scale data. Broader-ranging issues such as acid rain, the ecological decline of the Chesapeake Bay, and regional runoff of pesticides are examples of such regional-scale environmental problems. They are especially interesting, because any extension of scale requires a redefinition of endpoints, the introduction of new uncertainties, and integration of the assessment with different societal concerns and management objectives.

For the Future

We believe the development of risk analysis concepts and tools will lead to better and more defensible future decisions concerning environmental protection. Use of clearly defined endpoints will lead to more consistent results and will help the regulatory decision makers, the public, and the regulated industries to understand the implications of the assessment results. A better basis for understanding and consensus will be possible if uncertainties are acknowledged and results are expressed as effect probabilities or distributions, rather than as absolutes. At present, the advocates of risk assessment are still a minority within the environmental regulatory community. We have shown the skeptics that ecological risk assessment is possible; we must now convince them that it is also necessary. ornl

Glenn W. Suter II joined ORNL's Environmental Sciences Division in 1975, after completing a Ph.D. degree in ecology at the University of California at Davis. He received ESD's Scientific Achievement Award in 1987.

Lawrence W. Barnthouse came to ORNL in 1976, with a Ph.D. in biology from the University of Chicago. He is leader of ESD's Environmental Risk Group.

Carolyn Hunsaker is a research scientist in ESD's Regional Resources Group. She received a doctorate in environmental science and engineering from the University of California at Los Angeles. She is leader of the Regional Ecological Risk Assessment Project funded by the Environmental Protection Agency. She is secretary of the national board of the Association for Women in Science.

The National Environmental Research Park at Oak Ridge

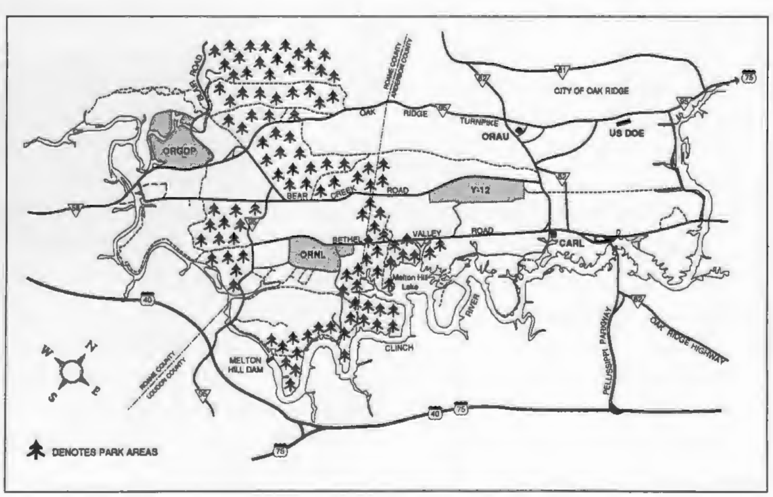
Where can you go to study the natural changes in a stream as it grows from a trickle to a torrent, flows from hills to valleys, courses through pine forests and over limestone ledges—with virtually no interference and no pollution by human activity? Few large land areas anywhere in the world are suitable for such an investigation. Recognizing the need for preserving some land in its natural state for environmental and ecological research, the Department of Energy (DOE) in 1980 designated nearly 13,000 acres of the Oak Ridge Reservation for use as a National Environmental Research Park (NERP). In April 1989, the NERP was also selected by the United Nations Educational, Scientific, and Cultural Organization (UNESCO) as a unit of the Southern Appalachian Biosphere Reserve. This makes the research park part of a global network of sites set aside for international cooperative environmental research.

As a region representative of the temperate broadleaf forests of the Southern Appalachians, the research park serves as an "outdoor laboratory" and a useful study control area for many types of ecological and environmental research. In conjunction with the major data bases and information centers located at ORNL, the NERP provides an environmental research facility that is equalled at few other places in the United States.

The research park is one of the national DOE user facilities at ORNL and is open to qualified researchers in any scientific field, provided the work on the park lands is compatible with the DOE energy research, development, and production programs conducted at Oak Ridge. NERP Manager Pat Parr, of the Environmental Sciences Division, coordinates and monitors the research park's activities.

A recent, and very successful, innovation at the NERP is the Ecological and Physical Sciences Study Center, which has become very popular with local educators. Although the research park has always served as an educational resource for field trips by visiting groups of students, the Study Center goes a step further by providing study modules designed for students of grades 4 through 12 and presented by ORNL staff members or highly qualified professional teachers. When the Center was first opened in 1984, study modules in the life sciences were presented to about 125 local students and teachers. Between 1984 and 1988, the Center presented study modules in a wide variety of science subjects to nearly 20,000 local participants (see article on p. 75). The Study Center is helping to enhance the quality of science education throughout East Tennessee.

"The Department of Energy designated nearly 13,000 acres of the Oak Ridge Reservation for use as a National Environmental Research Park."



Regulatory Analysis

By Frances E. Sharples

Protection of the environment became a focus of intense public and Congressional interest during the 1970s. Public awareness of both the hazards of pollutants and toxic substances and the damage caused by their releases to air, water, and soil increased rapidly. The U.S. Congress created programs designed to correct existing damage and prevent further environmental degradation in a series of major environmental protection statutes: the Clean Air Act (CAA) of 1970; the Federal Water Pollution Control Act of 1972, later amended to become the Clean Water Act (CWA); the Safe Drinking Water Act (SDWA) of 1974; the Toxic Substances Control Act (TSCA) of 1976; the Resource Conservation and Recovery Act (RCRA) of 1976; and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980, better known as Superfund. Each piece of legislation authorized major new federal regulatory programs to be administered and enforced by the Environmental Protection Agency (EPA), which was itself created by legislation signed in December 1970.

On the whole, the results of this decade of environmental legislation have been positive, and environmental quality is improving across the nation. The regulatory programs designed to implement these laws are, however, often complex mazes of rules, regulations, procedures, and requirements that change frequently as EPA implements statutory schedules and responds to periodic legislative amendments. The complexity and frequent change make compliance with environmental regulations a continuing challenge for those who are regulated.

Agencies of the federal government, such as the Department of Energy, are subject to most of the same environmental compliance requirements as the private sector. Understanding and meeting regulatory requirements, therefore, has become a major interest to DOE. The Environmental Compliance Group in the Environmental Analyses Section of the Environmental Sciences Division (ESD) at ORNL provides technical support and assistance for many of DOE's environmental compliance activities. Our major sponsor is the Office of Environmental Guidance (OEG) in the Office of the Assistant Secretary for Environment, Safety, and Health. Other DOE-funded programs and offices that currently sponsor our activities include the Hazardous Waste Remedial Actions Program, the ORNL Remedial Action Program, the Office of Civilian Radioactive Waste Management (OCRWM), and the Diffusion Process Permanent Shutdown Engineering Feasibility Study Group at the Oak Ridge Gaseous Diffusion Plant (ORGDP). We also perform tasks related to environmental regulations at the request of other federal agencies, such as the Navy and the Air Force.

How We Help

Our group provides a variety of services related to regulatory compliance. With funding from OEG, we maintain and continually refine a system to track and analyze regulatory initiatives. We generate a weekly electronic report identifying items in the Federal Register that are of particular importance to DOE and our other federal sponsors. A monthly update document series that we produce tracks important new regulations through all the phases of rule promulgation. We also produce a series of environmental guidance program reference books, which contain the texts of environmental statutes and their implementing regulations. These books are revised regularly to incorporate final regulation changes, so that users have the most complete and up-to-date versions.

We analyze in depth new rules that will have particularly important or widespread impacts on our sponsors. Because environmental regulations often encompass technical information from many different fields (e.g., hydrogeology, environmental engineering, and health and environmental risk assessment), our regulatory analysis team includes members of several other divisions having expertise complementing that of our own environmental scientists. Members of the Health and Safety Research Division and the Chemical Technology

Division work closely with us in detailed analyses of the technical aspects of regulations and in defining their potential impacts on DOE's diverse facilities and programs. The contributions of this team are particularly important at the "proposed rule" stage in the regulatory process, when the regulated community (including DOE) is to comment on the regulatory agency's proposals. Our technical analyses often provide DOE with the background information needed to formulate its responses to EPA initiatives.

A Broader Focus

Broader regulatory compliance tasks that are focused on specific facilities and programs comprise another category of group activities. In FY 1988, for example, we analyzed the applicability of hazardous waste regulations to high-level waste and evaluated strategies that the OCRWM might consider in developing policy for a geologic repository. We also analyzed the applicability of federal environmental regulatory requirements to the permanent shutdown of the ORGDP enrichment facilities in association with the Engineering Feasibility Study Group. We recommended ways to achieve regulatory compliance to assist ORGDP in determining costs and schedules for placing the plant in the "safe storage" phase. In addition, we performed environmental audits of Air Force bases, again including team members from a number of other ORNL divisions under ESD leadership. A final, very important aspect of our work is providing assistance in training DOE field office and contractor staff in both the basics and the details of environmental compliance, particularly focusing on the highly complex issue of hazardous waste. ornl

Fran Sharples, who has been an ORNL staff member since 1978, is leader of the Environmental Compliance Group in the Environmental Analyses Section of ORNL's Environmental Sciences Division. She has a Ph.D. degree from the University of California at Davis and has been an EPA Environmental Science and Engineering Fellow and a Congressional Science and Engineering Fellow.

Fran Sharples (left), Fred Baes, and Kathy Brown discuss documents recently produced by ESD's Environmental Compliance Group.

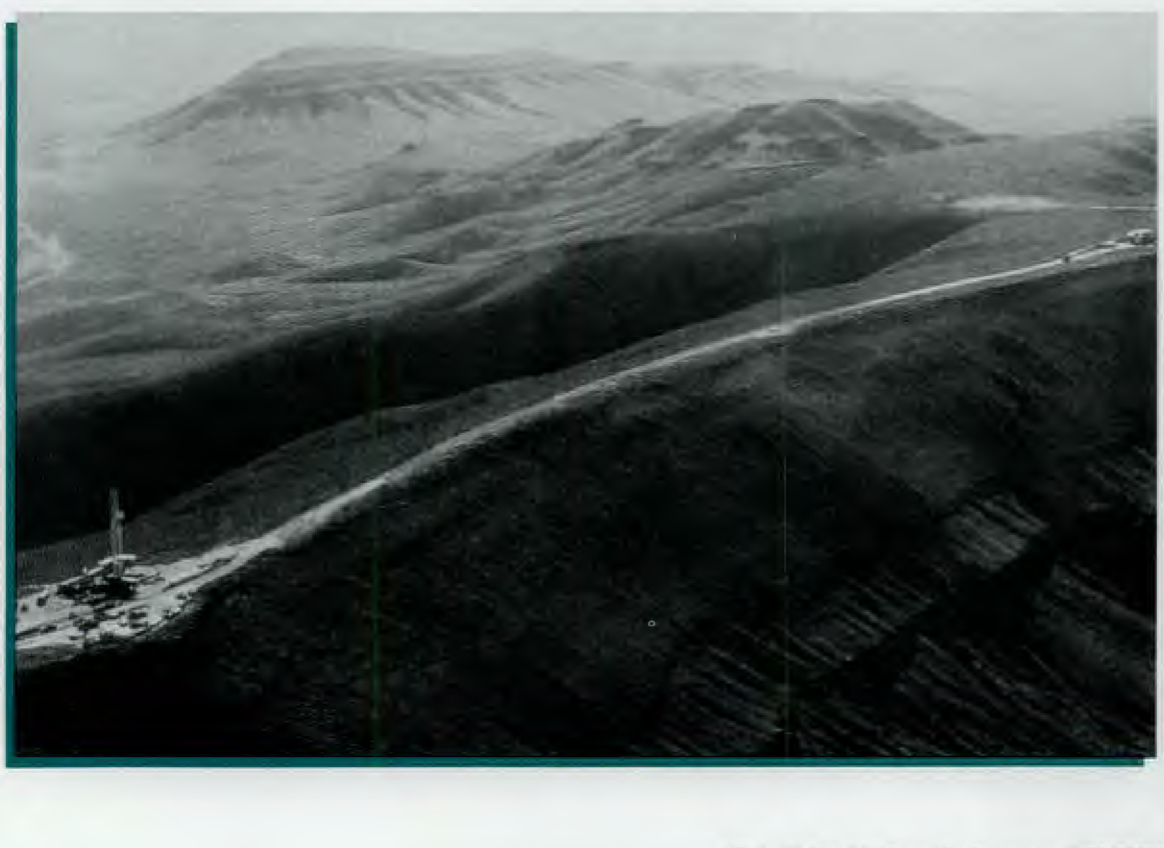
Geoscience and High-Level Radioactive Waste

By Gary K. Jacobs and Stephen H. Stow

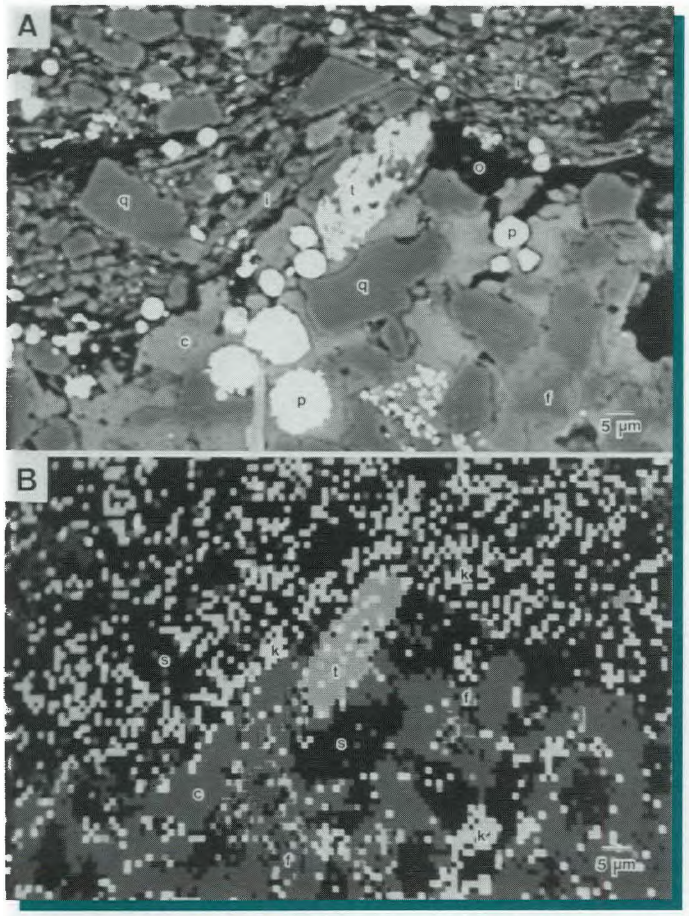
One of the most critical tasks facing geoscientists today is the safe disposal of significant quantities of high-level radioactive wastes (HLW) being produced by commercial nuclear reactors and nuclear defense programs in the United States. The problem is not new, however. At a 1955 meeting of prominent scientists and engineers at Princeton University, the participants concluded that HLW should be disposed of in deep geologic formations. Since then, several studies comparing alternatives to deep geologic disposal have reached the same conclusion as the Princeton group. Although the specific geologic formations investigated have changed over the years, the U.S. Department of Energy and its predecessors have followed this directive in searching for a permanent HLW disposal site. Yucca Mountain, on the Nevada Test Site, is the area now being studied.

Geologic Repository

A geologic repository will consist of a series of mined cavities at depths greater than ~300 m in stable rock formations. The wastes will be encapsulated in corrosion-resistant metallic canisters, placed in the cavities, and surrounded (in some cases) with a clay-rich packing material to help slow the migration of water to the canisters and reduce the mobility of contaminants released when the canisters fail. According to current regulations, (1) the canisters must last from 300 to 1000 years; (2) after failure, radionuclides must be released from the waste packages at a rate of <0.001% of the total inventory each year; (3) groundwater flowing through the repository must take at least 1000 years to reach the accessible environment (defined as the atmosphere, land surfaces, surface waters, etc., or a horizontal distance in the



Geologic site-charaterization studies for the high-level waste repository are under way at Yucca Mountain on the Nevada Test Site.



Examining the microstructure, mineralogy, and chemistry of shales allows an evaluation of their ability to slow the migration of radionuclides. (A) Backscattered electron micrograph of a Chattanooga Shale shows the phases illite (i), organic matter (o), quartz (q), feldspar (f), pyrite (p), calcite (c), and a titanium-bearing phase (t). (B) Computer map of the same area shows the distribution of elements: potassium (k) in feldspar and illite, silicon (s) in quartz, iron (f) in pyrite, calcium (c) in calcite, and titanium (t) in the unidentified titanium phase.

subsurface 5 km from the edge of the repository); and (4) the total release of radioactive material from the repository to the environment must remain below specified levels for 10,000 years.

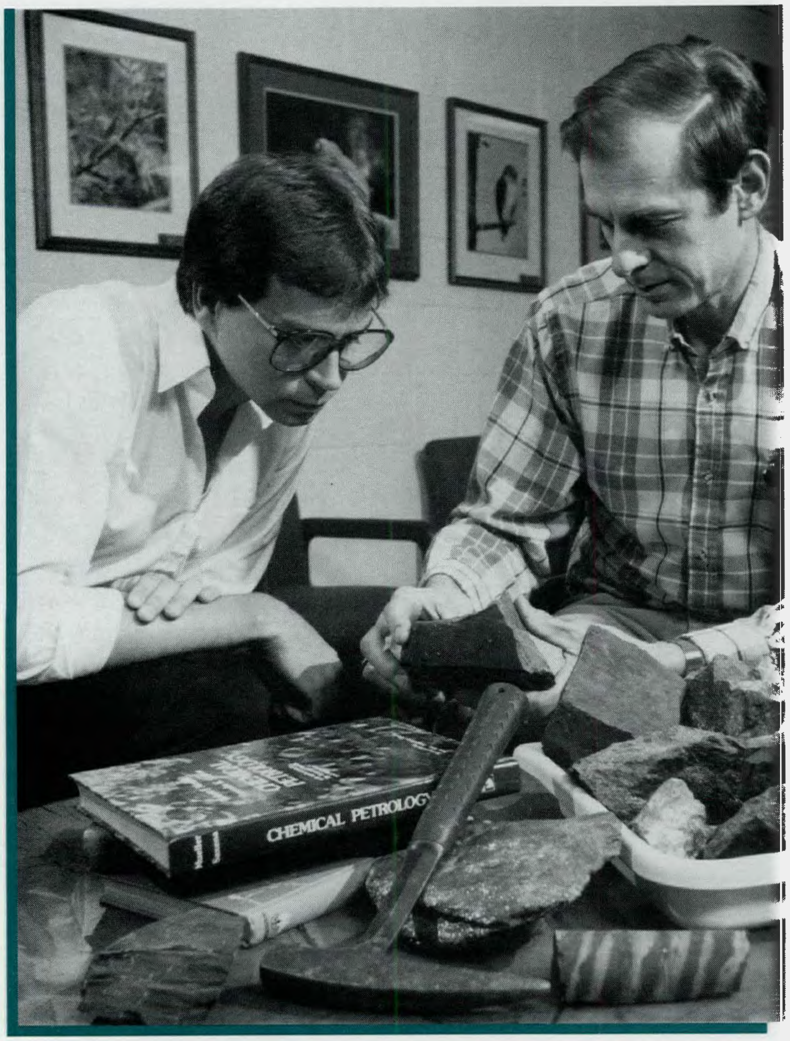
In the most likely scenario for the release of contaminants, water flows into the repository, corroding the canisters, dissolving the waste form (spent fuel rods or glass cylinders), and transporting the contaminants in groundwater. Thus, the best sites for a repository have only a little groundwater, which moves slowly through long flow paths to the accessible environment. These flow paths should contain highly sorbent minerals that act as scavengers for radionuclides. In addition, the groundwater should have geochemical properties that inhibit corrosion, waste form dissolution, and radionuclide mobility.

Over the years, DOE has considered several rock types (e.g., basalt, salt, tuff, granite) as repository candidates. However, in December of 1987, the U. S. Congress directed DOE to concentrate on only one site, Yucca Mountain, which is located on the Nevada Test Site. The repository would be located several hundred meters below the surface yet well above the groundwater table in tuffaceous deposits (silica-rich volcanic rocks) that are generally highly sorbent for radionuclides.

Sedimentary Rock Studies

During the past five years, researchers at ORNL have studied sedimentary rocks to evaluate their potential as repository hosts. Because of the 1987 directive, the program at ORNL has shifted its emphasis to provide scientific support for the Yucca Mountain site. Fortunately, many of the techniques and analytical tools developed during this time are transferable to programs at Yucca Mountain; should there be a need to consider sedimentary rocks for waste disposal sites in the future, the data generated by ORNL researchers would be directly applicable.

The original objective of the Sedimentary Rock Program (SERP) was to investigate sedimentary rocks (shale, sandstone, chalk, carbonate, anhydrite) for their potential as repository hosts; this work was done by staff members from the Chemical Technology and Environmental Sciences divisions. Ranking the types of sedimentary rock with respect to geology, geochemistry, hydrology, thermal performance, rock mechanics, natural resources, and waste package degradation suggested that, of the sedimentary rock types, shale offers the greatest potential for the safe isolation of radioactive wastes. Shales are composed primarily of clay minerals (smectite, illite,



Gary Jacobs (left) and Steve Stow examine various rock types that could be useful for the disposal of high-level radioactive wastes.

kaolinite), having lesser quantities of phases such as quartz, calcite, pyrite, feldspar, and organic matter. Because the characteristics of shales span a rather broad range, more detailed studies of their hydrologic, geochemical, and thermomechanical properties were undertaken to identify possible relationships between the geologic attributes of shales and the safety aspects of a repository.

The major emphasis of our shale studies has been in geochemistry, because of the expertise of the researchers in this field. The geochemical factors investigated at ORNL include groundwater chemistry, mineralogy, sorption, and contaminant transport. These four aspects are important in evaluating canister corrosion, the dissolution and release of radionuclides from waste forms, and the subsequent migration of contaminants away from the repository in this medium.

ORNL's Shale Studies

Information on the chemistry of groundwaters typically associated with shales has been obtained from literature sources, U.S. Geological Survey data bases, experimental studies, and computer modeling. Karen Von Damm, a geochemist in ESD, found that shale groundwaters range from dilute, potable waters to concentrated brines. The groundwaters are generally mildly basic, a characteristic desirable for emplacing metal canisters. Results from computer modeling of chemical interactions between the groundwaters and the shales suggest that dissolution of halite (common salt) from the shales may have been important in the past development of their groundwater chemistry. In addition, it is probable that the groundwaters have low concentrations of oxygen, also favorable for inhibiting corrosion as well as for minimizing the mobility of some radioelements (e.g., uranium).

The mineralogy and microstructure of the shales have been investigated by Suk Young Lee and Kirk Hyder of ESD. They used optical microscopy, X-ray diffraction, scanning electron microscopy, and high-resolution transmission electron microscopy to identify and characterize the individual phases in shales. Studies of the organic matter in shale were done by Patience Ho of the Chemistry Division and Necip Güven and Charles Landis at Texas Tech University. The information obtained on the mineralogy, organic chemistry, and microstructure helps us evaluate (1) potential changes in the shales as a result of decay heat from the waste packages (temperatures up to 250°C); (2) the ability of various minerals to sorb radionuclides and slow their migration; (3) potential changes in groundwater chemistry via reactions with shale minerals; and (4) the effects of microfractures and micropores on the flow of groundwater through shale.

One of the most desirable characteristics of shale as a candidate host rock is its ability to sorb radionuclides. Sorption refers to the process by which some dissolved radionuclides become "stuck" to solid phases as the groundwater moves through the rock, thus retarding the movement of the radionuclides. By migrating more slowly than groundwater, the radionuclides have an opportunity to decay to lower concentration levels before reaching the surrounding environment.

Bob Meyer, Bill Arnold, Dave O'Kelley, and Faith Case of the Chemistry Division have studied the sorption of various radionuclides onto shales. Samples of shale are contacted with solutions containing small amounts of the radionuclide of interest, and the amount of radionuclide sorbed is measured. Factors found to influence the degree of sorption onto shales include the (1) salinity of the groundwater, (2) contact time between the shale and the radionuclide-bearing solution, (3) ratio of water to rock, (4) temperature, and (5) amount of organic matter in the rock. Although each radionuclide behaves somewhat differently, we have found that, in general, shale is highly sorbent and should act as a good scavenger for radionuclides.

The time scale that must be considered in evaluating the performance of a repository is thousands of years. Therefore, we must use reliable computer models to predict groundwater flow and radionuclide migration through the rocks. Models for this purpose combine data from hydrology, geochemistry, geology, and engineering studies; the predictions are only as reliable as the models and data used. Vijay Tripathi and George Yeh of ESD have been working to improve an existing model (HYDROGEOCHEM) by incorporating important hydrologic and geochemical processes into the model as realistically as possible. As more of the actual hydrologic and geochemical processes involved in the migration of contaminants are included in the model, more reliable predictions will be possible.

Of course, as models are made more complex, the time and expense of running them on existing computers increases. Therefore, our scientists have worked both to enhance the model and to improve its computational efficiency. Much of the shale-related geochemical information will be integrated in a model that can evaluate the migration of radionuclides through various shales.

As our emphasis shifts from performing detailed investigations of shales to providing scientific support for the Yucca Mountain site, the experience and knowledge gained from our activities in the past several years will be invaluable. Our program, now referred to as the Geoscience Technology Support (GETS) Program, is now seeking solutions to fundamental hydrogeochemical problems that will help resolve site-specific issues being addressed at Yucca Mountain and will add to our basic knowledge of groundwater geochemistry, mineralogy, sorption, and contaminant transport. It will also help ensure that radioactive wastes existing today and those produced in the future will be safely isolated. ornl

Gary Jacobs is leader of the Geochemistry Group in the Geosciences Section of the Environmental Sciences Division. He received his Ph.D. degree in geochemistry from Pennsylvania State University in 1981 and joined the ORNL staff in 1983.

Stephen Stow is head of the Geosciences Section. Before joining ORNL in 1980, he was professor of geology for 11 years at the University of Alabama. He received his Ph.D. degree in geochemistry from Rice University.

Groundwater Modeling

By Laura Toran and D.K. Solomon

Concern about whether our water is safe to drink has increased since the recent discovery that improper waste disposal practices and other anthropogenic and natural hazards threaten to contaminate groundwater and surface water. In response to this concern, researchers at ORNL are developing and using computer models to improve our understanding of natural and contaminated groundwater systems.

Models describing groundwater flow and contaminant transport enable scientists to learn more about problems of groundwater supply and contaminant travel times. Using a computer, a modeler can explore various environments, stresses, complex systems, and long-term processes that would otherwise be impossible to study within a human life span. Model analysis helps hydrogeologists plan field studies and interpret field data, as well as to make predictions and study hypothetical problems. Thus far, our models have proven to be more successful in studying groundwater flow problems than in the more complex interrelationships of groundwater flow and contaminant transport.

Laura Toran models groundwater flow.

A Misunderstood Tool

Computer models remain one of the most misunderstood tools in hydrogeology, mainly because of unreasonably high expectations about the ability of models to make predictions, coupled with the urgent need for accurate predictions. When researchers use models to solve groundwater contamination problems, caution is needed in interpreting model results. Computer models in which we have varying degrees of confidence touch all of our lives. For example, meteorologists use models to predict the weather—with somewhat limited success, as many a soggy picnicker knows. Engineers use models (sometimes using equations similar to those describing groundwater flow) to test new designs; they can validate the models by building and testing the equipment.

Groundwater models are not as amenable as these examples to testing or validation. Groundwater moves slowly, and predictions may be made for many years in the future—as opposed to the weatherman's weekend forecast, which provides more immediate feedback on reliability. The geology of a groundwater system cannot be as easily reconstructed as, for example, an airplane wing, making it difficult to model the basic components. Furthermore, groundwater models that include contaminant transport are currently limited by the lack of knowledge about the physical and chemical processes that control transport. How to simulate numerically the apparent dispersion of contaminants in a porous medium is debatable, and researchers are just beginning to understand the complex chemical and biological reactions occurring in groundwater.

As a result, groundwater models are shaped to a great degree by the individual modelers, who must understand these limitations thoroughly. Like a piece of music played by two different musicians, the results produced by two different modelers can be quite different. Furthermore, the thought processes that go into constructing a model are probably even more important than the results.

Interdisciplinary Models

In ORNL's Environmental Sciences Division (ESD), our research with groundwater models is based on a broad range of expertise, including the development of computer codes, field evaluation, and model use and interpretation. Hydrogeologists using the models also work with staff members from a variety of disciplines including mathematics, computer science, geology, and geochemistry.

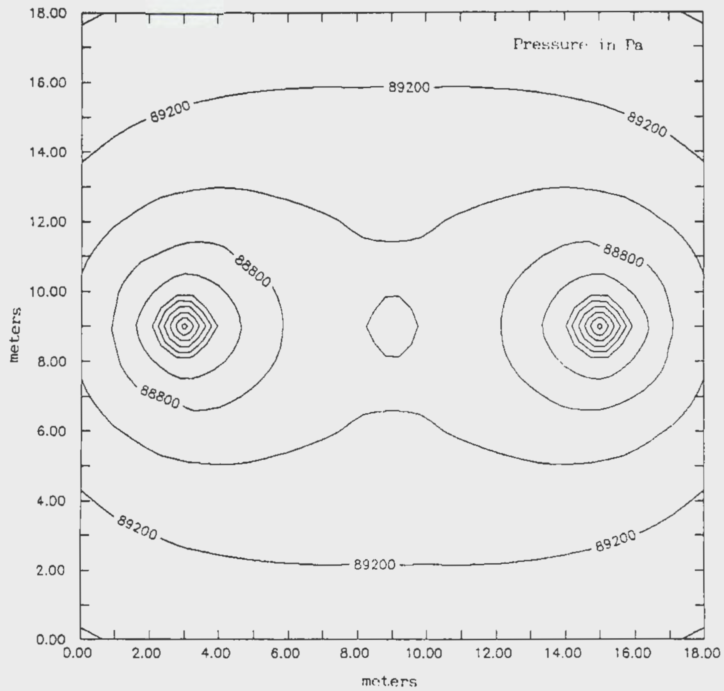
In the field of model development, ESD scientists have led the way in combining groundwater flow with geochemical processes. A new computer code (HYDROGEOCHEM, developed by G. T. Yeh and V. S. Tripathi of ESD) is designed for computational efficiency and is the only groundwater model that simultaneously solves for multiple chemical processes, including adsorption, precipitation, complexation, oxidation-reduction, and some biologically mediated reactions. In addition, it examines both saturated and unsaturated groundwater flow conditions.

ESD staff members have used computer models to study the effect of rainfall on water levels in waste disposal trenches on the ORNL site, to predict biodegradation rates of organic contaminants, and to find equations that describe bacterial transport in groundwater. Another area of research deals with improving field techniques for measuring model parameters. We are developing instrumentation to measure groundwater velocity, studying the effect of fracture orientation on preferred groundwater flow directions, and exploring different techniques to measure aquifer permeability in "tight" rock formations.

Soil-Venting Model

An example of the usefulness of our interdisciplinary approach to modeling is the recent development of a new model used to design an in situ soil venting system for removing volatile organic contaminants spilled at a Department of Defense facility. A computer model was needed to test various treatment schemes, but no codes were available that could calculate transport of contaminants in a compressible fluid such as air. Relying on our experience in developing computer codes for contaminant transport in water, we adapted the unsaturated transport equation in the 3DFEMWATER code (developed by G. T. Yeh) to relate air conductivity (the ability of a vapor to travel through a porous medium) to air pressure (analogous to the degree of saturation). The existing numerical algorithms for solving the equation were used, and the FEMAIR code was completed.

Kip Solomon measures conductivity of vapors through the soil at the Hill Air Force Base in Utah.



Computer simulation showing pressure drop in the two-well venting scheme used at Hill Air Force Base in Utah.

The next step was to find a way to measure the conductivity of vapors through the soil at the field site. The use of parameter values obtained from the specific site is critical to a model's usefulness. Techniques for measuring the conductivity of water were adapted for measuring the conductivity of air. A section of a well at the site was isolated using inflatable packers, air was injected under pressure into the packed-off interval, and the rate of pressure dissipation was measured to calculate air conductivity of organic vapors (see photo on previous page).

Using field parameters and other site data describing the physical geometry, we constructed a model to simulate removal of the vapor phase contaminants for different well configurations and air pumping rates. The recommended scheme that resulted from our modeling studies was to put barriers at the surface and to space the injection and vent wells about 6 m to 12 m apart to maximize air flow through the contaminated region. The venting scheme, which reduces the atmospheric pressure at the field site (as shown in the computer simulation on this page), is currently in operation at the Hill Air Force Base in Utah (see article on "Hazardous Waste Technology Programs" in the *Review*, No. 4, 1988). The system is monitored, and any discrepancies between model predictions and field results will be used to refine the preliminary model.

This example illustrates two of the scientific challenges facing groundwater modelers. First, we need physical data to describe contaminant transport. Controversies surrounding contaminant dispersion and groundwater chemical reactions must be resolved. Second, we need the means to validate models with field data. In general, the sophistication of the models has increased faster than our ability to make appropriate validating field measurements.

The ultimate goal in modeling is, after all, to learn more about the system, not to have a computer tell us what to do about our groundwater problems. Scientists will continue to have the responsibility of questioning model results and developing new techniques for better understanding our groundwater systems. ornl

Laura Toran is a research associate in the Environmental Sciences Division. She joined ORNL as a Wigner Fellow in 1986 after obtaining a Ph.D. degree from the University of Wisconsin.

D. K. Solomon has been a research associate in the Environmental Sciences Division since 1985. He holds an M.S. degree in geology from the University of Utah and is working on a Ph.D. degree at the University of Waterloo, Canada.

Water, Rocks, and Waste Disposal

Hydrogeologic research and computer modeling lead to improvements in waste disposal.

By Dale D. Huff and Stephen H. Stow

Evaluating disposal practices for hazardous chemical and radioactive waste is a major part of the geosciences at ORNL today. Geoscientists are characterizing past disposal sites and devising ways to clean them up and ensure that present and future disposal operations are properly undertaken. To carry out this work, the relationships among buried waste materials, the rock substrate in which disposal has occurred, and the water in contact with the substrate must be understood (see figure on next page).

Because water is present in most waste disposal sites, complicated problems can arise. Water can dissolve or suspend many different materials, including wastes from a disposal site, and move these materials through the soil and rocks, making them available to tree roots, surface streams, or, in the worst case, aquifers from which well water is drawn for drinking. Thus, our investigation of the ways in which water reacts with waste products and the mechanisms by which it transports contaminants through rocks forms an important and highly technical aspect of earth science studies at ORNL.

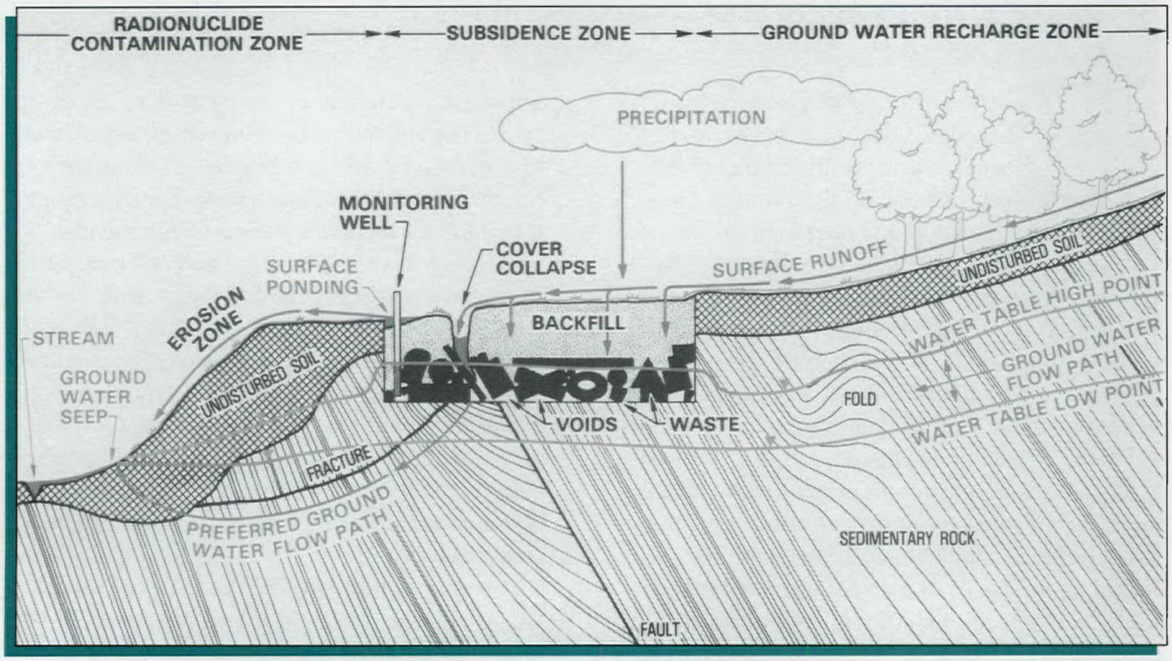
In the Environmental Sciences Division (ESD), we have several research projects that involve water, rocks, and waste disposal. The research embraces field work (e.g., basic mapping and hydrologic studies), supplementary laboratory investigations, and computer modeling to assess and simulate conditions observed in our field and laboratory studies. ESD staff members are also involved in basic research on the geologic, hydrologic, and geochemical issues linked to disposal of high-level radioactive wastes and other disposal operations at Department of Energy sites throughout the country.

Controlling Water Contamination

Water is important in many waste disposal considerations. First, water can leach out and transport contaminants. Second, water is an important medium in the pathway between disposal sites and the surrounding plant and animal life. In parts of the United States (such as the Southeast), where rainfall greatly exceeds evaporation losses, there is a greater likelihood that waste constituents will be leached and transported. Because of our heavy rainfall in this area, ORNL is an excellent site for studying these water-waste relationships. There are two basic approaches to controlling water contamination from waste disposal operations: (1) create a waste form that does not react with water and (2) use engineered barriers to prevent water-waste contact. At ORNL, both options are being studied, sometimes in combination.

A component of ORNL's Low-Level Waste Disposal Development and Demonstration Program (see article in the *Review*, No. 4, 1988) includes the design of disposal technologies that exclude or minimize intrusion of water into the waste. The tumulus concept being demonstrated in ORNL's Solid Waste Storage Area 6 is an example. Wastes are stored in a solidified form within concrete casks on a reinforced concrete pad (tumulus). When the pad is fully loaded, the mass of wastes is covered with fill material and a multilayered cap of very low water permeability. The pad has a liquid-collection system that is part of a comprehensive performance evaluation network that includes groundwater monitoring wells. Long-term investigations of hydrologic conditions at and around the site will enable us to evaluate the effectiveness of the technique and to identify any design modifications needed.

At older, shallow-land disposal sites, we are studying the hydrologic processes that mediate migration, with the goal of designing effective measures to stabilize these processes. Tracers are used to explore the dominant flow pathways and the temporal variations in releases. For example, a new technique is being developed to determine how long a water sample has been isolated from atmospheric exchanges. This tracing method compares the ratio of helium isotopes in groundwater with the known ratio of helium isotopes in



Water interacts with low-level waste materials as it flows from the surface into shallow land burial trenches and along fractures in deformed bedrock.

atmospheric water vapor. The decay of tritium in groundwater during subsurface water movement distorts the helium ratio when tritium decays to the lighter helium isotope (^{3}He). Our measurements have identified groundwater that has been separated from the atmosphere for as little as six months. Young groundwater like this is sometimes associated with preferred flow pathways.

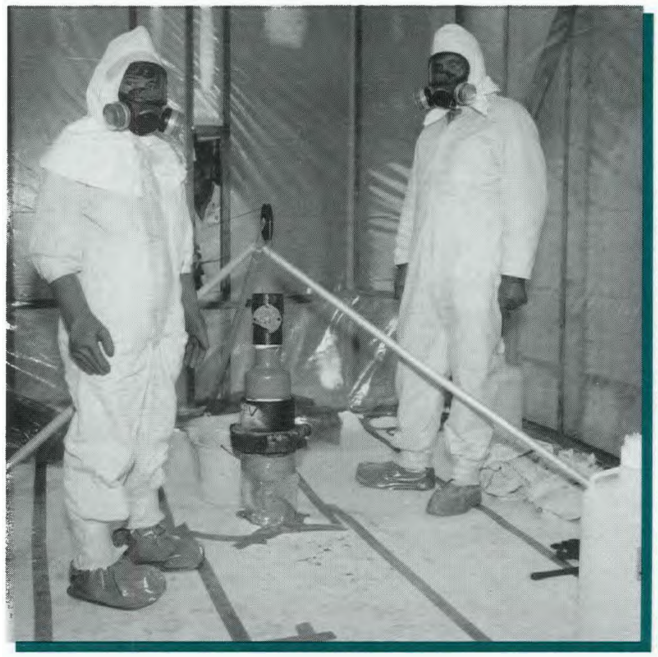
Waste-derived tritium is another tracer in streams. Variations in tritium concentration and mass flow in first-order (i.e., the smallest) streams in one of our newer disposal areas have shown that nearly two-thirds of the annual contaminant flux comes from shallow subsurface flow pathways, mainly during storms. This runoff moves rapidly through the region above the water table, rather than recharging the groundwater. Existence of this lateral-flow pathway in the unsaturated zone has significant implications for developing monitoring strategies and for planning corrective actions.

We are also applying water-flow simulation models to the study and analysis of contaminant transport. A three-dimensional, finite-element, groundwater flow model is being used to study the influence of human excavations on pathways and rates of natural groundwater flow. Studies using dye tracers show that groundwater movement can be accelerated as water moves through the permeable backfill surrounding buried utility lines. Where such backfill intersects the saturated zone, it acts as a drain and distorts natural flow patterns and rates. Simulations are being used to examine ways to control this flow.

Once groundwater contaminants emerge in streams, estimates of their movement and changes in concentrations during downstream transport are needed. The Streamflow Synthesis and Reservoir Regulation Model, developed by the U.S. Army Corps of Engineers, has been adapted here to simulate contaminant transport in surface-water systems from area or point-source inputs, including accidental spills. Dye studies and comparisons with measured flows have been used for model calibration, and the model now serves as a tool both for managing contaminant releases and for defining the data collection needs for basin-wide evaluation of contaminant transport.

Rock Studies

Groundwater is responsible for most contaminant migration away from a disposal site. Pore spaces in the rock surrounding a disposal site allow the groundwater to flow through, and water-rock reactions affect the mobility of dissolved contaminants. The physical characteristics of the rocks that surround the waste disposal area control the movement of the groundwater; for instance, the fractures, faults, and folds in the strata create preferred pathways for groundwater flow. To predict contaminant mobility—and the possible interaction of contaminants with the surrounding environment—an understanding of the physical and chemical nature of the rocks is essential. Without this knowledge, a full interpretation of results from hydrologic studies is impossible, and geochemical reactions cannot be anticipated.



ESD geologists Steve Haase (left) and John Switek prepare to sample a well for studies of radionuclide migration in groundwater near ORNL disposal sites.

At ORNL, we conduct field studies to reveal details of the rock fracture systems—chiefly in shale and sandstone—through which groundwater moves. Fractures, the result of mountain-building stresses imposed hundreds of millions of years ago, largely control the movement of contaminated groundwater. Multiple sets of fractures exist, many of which are interconnected, making their detailed structural analysis very difficult.

Groundwater also moves through solution cavities in carbonate-rich strata. These cavities are formed when groundwater chemically dissolves the rocks, creating relatively large pathways through which groundwater can flow rapidly. Learning about the distribution of rock types, as well as their tendencies to fracture or form cavities, is an essential part of the on-site work. Methods for filling voids and fractures with impermeable grouts are being investigated as a means of controlling groundwater movement and reducing migration of contaminants from waste-disposal sites.

Although some rocks are soluble in water, others have the ability to chemically retard contaminants and effectively remove some of them from the groundwater. In addition, the rocks themselves may alter the chemical composition of the groundwater, thereby influencing its ability to transport contaminants. Techniques that alter the physical and chemical properties of rocks—for example, in situ vitrification (immobilizing radioactive waste by electrically melting it with soil to form a massive glass block)—are being studied as methods of controlling contaminant migration.

Our research at ORNL has shown that all of the inseparable hydrological, geological, and geochemical relationships of disposal sites must be considered in evaluating and improving waste disposal practices. We are making progress in understanding these relationships.

Dale D. Huff, head of the Environmental Engineering and Hydrology Section of the Environmental Sciences Division, came to ORNL in 1974. He holds a Ph.D. degree in hydrology from Stanford University and received an ORNL Environmental Protection Award.

Steven H. Stow is head of ESD's Geosciences Section. He joined ORNL in 1980 after an 11-year tenure as a professor of geology at the University of Alabama. He holds a Ph.D. degree in geochemistry from Rice University and serves on numerous national committees dealing with waste disposal and the geosciences.

Radioactive Waste Disposal and Environmental Engineering

By E. C. Davis

Environmental engineering is the branch of traditional civil engineering that deals with air pollution, water supply and treatment, wastewater collection, treatment and disposal, and municipal and industrial solid waste management. At ORNL, work is ongoing in each of these areas; however, liquid and solid radioactive waste management and disposal offer special research and development challenges to the environmental engineer.

Since 1943, ORNL has used six areas totaling 57 ha for disposing of radioactive low-level solid waste. These areas contain about 200,000 m^3 of waste having an activity level of ~300,000 Ci. Shallow land burial in trenches and holes dug here has been the principal disposal method, and an estimated 730 rectangular trenches and 1400 disposal holes have been filled in Solid Waste Storage Area (SWSA) 5 and SWSA 6 alone. In addition, from 1955 to 1963 ORNL served as the Atomic Energy Commission's southeastern radioactive waste disposal site and received low-level waste shipments from across the southeastern United States.

Early observations and environmental monitoring in the 1960s at ORNL's solid waste disposal sites indicated that some radioisotopes were migrating from trenches to nearby surface streams after the trenches filled with water during the wet winter months. The water inundation and leaching of radionuclides have led to the design, construction, and evaluation of several demonstration remedial action projects: (1) trench covers of bentonite clay mixed with soil; (2) surface and groundwater (French drain) ditches to divert water

Metal boxes containing low-level radioactive wastes were formerly placed in trenches within designated solid waste storage areas.

Flow rates are carefully checked in surface water streams of Solid Waste Storage Area 6.

away from trenches and to suppress the seasonally fluctuating water table; (3) dynamic compaction of closed trenches to reduce cover subsidence; and (4) in situ grouting of trench contents to fill void space and prevent water inundation.

Each of these experimental remedial measures has involved the coordinated efforts of environmental and civil engineers, geologists, hydrologists, and soil scientists. Each new project and follow-up evaluation has also generated new ideas that contribute to ORNL's site-wide Remedial Action Program, which is leading the effort to stabilize and close radioactive waste disposal sites.

Besides addressing the environmental issues linked to past waste disposal practices, ORNL's environmental engineers are improving present and future disposal practices. For example, the confinement of waste in reinforced concrete silos is being investigated as a replacement for unlined earth trenches and disposal holes. We have also helped design and construct a demonstration above-grade concrete storage pad (waste tumulus) for stacking sealed, waste-containing, concrete vaults. This facility makes waste isolation possible in lower-elevation regions where the water table is too shallow to permit below-grade disposal. Our engineers will also be involved in evaluating these new disposal demonstrations. ornl

Ed Davis joined the Environmental Sciences Division staff in 1977 and, three years later, received his Ph.D. degree in environmental engineering from Vanderbilt University.

Assisting him in his research are Della Marshall, Brian Spalding, Bill Boegly, Jr., and Sidney B. Garland.

Using an electronic tape, Ed Davis monitors moisture conditions in newly constructed storage silos for low-level solid waste, located in ORNL's Solid Waste Storage Area 6.

From Atoms to Humans

By Stephen V. Kaye

The Health and Safety Research Division (HASRD) was organized in April 1977 to investigate the impacts of energy technologies on human health. Until then, no single ORNL research division or program focused on human health as an endpoint. The Biology Division had conducted basic biological research, primarily on the mechanisms behind changes induced in experimental organisms by radiation and chemicals, and the Environmental Sciences Division (ESD) had concentrated on the effects of nuclear power and other energy technologies on the environment and ecological processes. HASRD was created to focus on research that complements these basic biological and environmental studies and to assess the impacts of environmental hazards on human health.

This type of research was being done in three different divisions: the research components of ORNL's former Health Physics Division, the Analysis and Assessments Section of ESD, and the Medical Radioisotopes Group of the Operations Division. When these were combined to form HASRD, we had an initial 110 regular staff and 65 guests to begin our exciting mission.

The reorganization brought together strong research programs in atomic, molecular, and radiation physics that received their core funding from the same DOE Office of Health and Environmental Research that supported many related applied programs in biomedical instrumentation, dosimetry, and nuclear medicine. Thus, the new division's research ranged from atoms to humans, and it was well-equipped to target health and safety issues (e.g., indoor air pollution) in a rapidly changing technological environment.

Strong Physics Program

Experimental and theoretical studies by HASRD's physicists have provided a strong core of basic research and brought honor to the division. Since 1977 five of our physicists have been named Corporate Research Fellows. Sam Hurst, who retired three years ago, was one of the first to be so honored. The remaining four—Loucas Christophorou, Bob Compton, Rufus Ritchie, and Jim Turner—continue very active HASRD research programs.

Our history of producing world-class experimental and theoretical research results in ionizing radiation physics and dosimetry has been a springboard to research in other areas. For instance, our years of studying electron mobilities in irradiated gases caused DOE to select ORNL as the lead laboratory for basic research on gaseous dielectrics. To date, we have organized a series of five international symposia on the topic, and our work has vastly improved scientific understanding of the physical properties determining the performance of gaseous dielectrics (e.g., in transformers and underground transmission lines).

Our work on fundamental processes in gases has resulted in an outstanding program in negative-ion physics and pioneering studies involving laser-initiated multiphoton ionization. This laser-based work, in turn, led to the development of resonance ionization spectroscopy (RIS), a technique for detecting a single atom of an element in a mixture of atoms of other elements—something like finding the proverbial needle in a haystack. Using RIS, we have detected a single stable cesium atom among 10^{19} atoms of all types in a gaseous sample, making our RIS device the world's most sensitive detector. This major achievement led to two patents, two I•R 100 awards, and the founding in 1981 of a spin-off company, Atom Sciences, Inc., in Oak Ridge. Numerous laboratories specializing in RIS have been established around the world, and HASRD has become recognized as a major center of laser expertise.

Radiation Dosimetry Leader

The division is also an international leader in internal radiation dosimetry. For over three decades, members of the Laboratory's original Health Physics Division and HASRD have served on key committees of the International Commission on Radiological Protection. Models and dosimetry methodologies developed at ORNL have been incorporated into the official radiological standards of countries around the world, and our

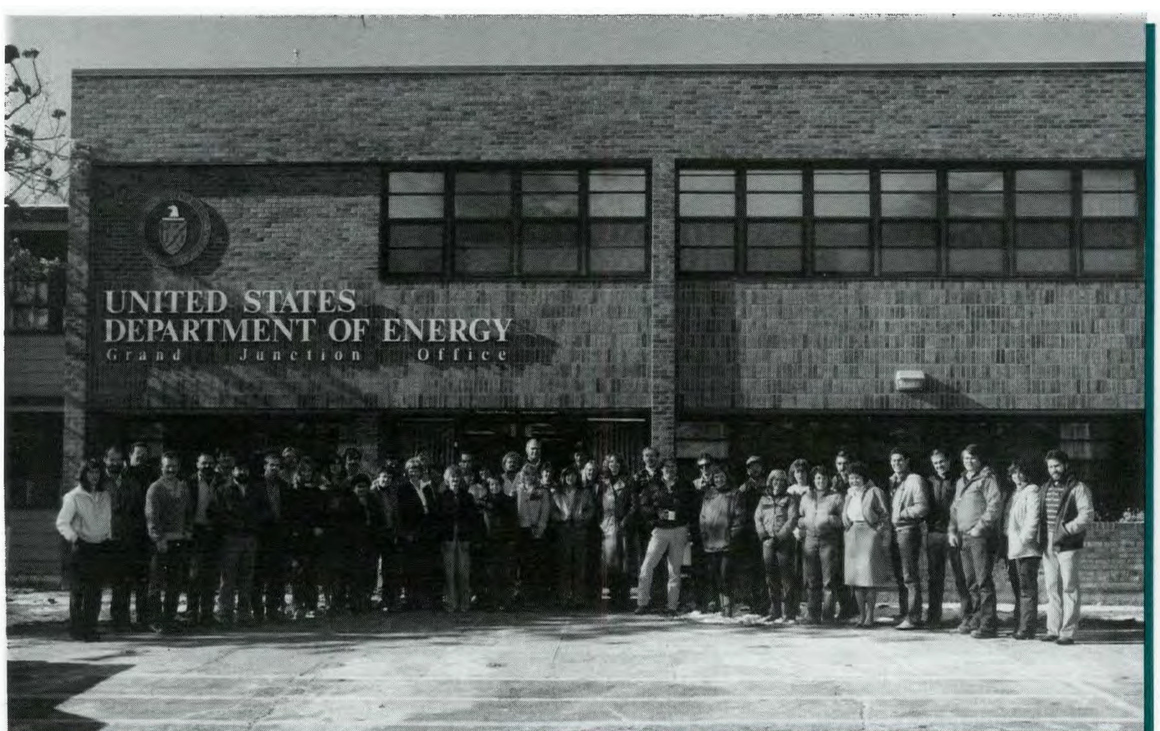
HASRD's basic research in atomic, molecular, and radiation physics leads to better instruments for measuring the impacts of energy technologies on human health.

methods for calculating radiological doses have been officially adopted and recommended by the Environmental Protection Agency (EPA), the Nuclear Regulatory Commission (NRC), and the Department of Energy (DOE)—a recognition of our contributions to the health protection area of the U.S. nuclear program.

A strong program in environmental radiation measurements complements the long-running internal radiation dosimetry and transport modeling work. In 1982, HASRD was designated the lead laboratory for DOE's Uranium Mill Tailings Remedial Action Program (UMTRAP), which identified properties contaminated with radioactive materials that require cleanup. This responsibility led to the 1983 establishment of office and laboratory facilities in Grand Junction, Colorado. Staffed by 60 full-time ORNL and subcontractor personnel, "ORNL West" has distinguished itself in environmental measurements and assessments. Although our UMTRAP responsibilities continue, the scope of the work has been expanded to include federal sites contaminated by toxic chemical wastes.

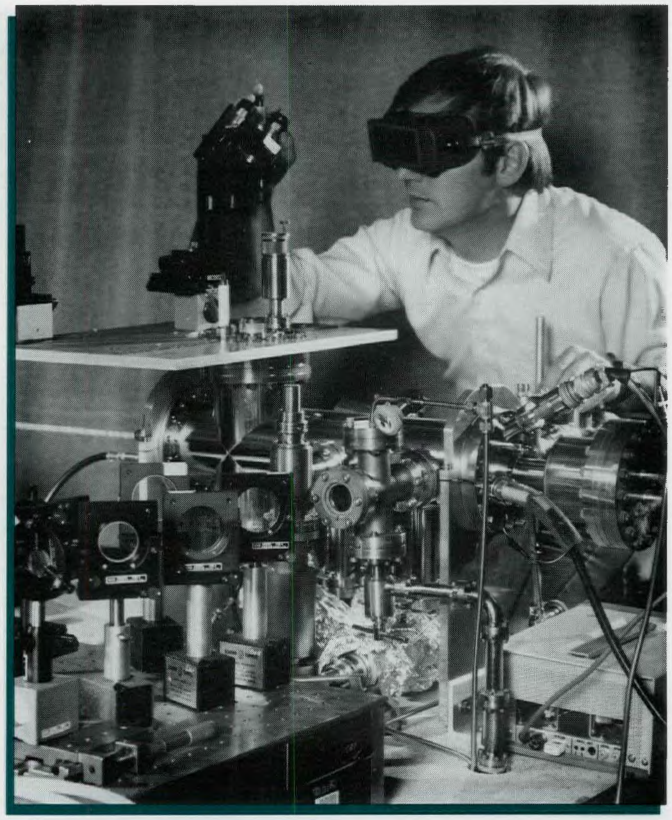
One of the predecessor divisions of HASRD, the Health Physics Division, established the Health Physics Research Reactor in 1963 as part of the Dosimetry Applications Research Center. Successful operation over the past 25 years has led to many achievements; as a result, HASRD reigns as the premier organization in personnel neutron dosimetry research. A new addition to the center has been the Radiation Calibration Laboratory (RADCAL), which is equipped with state-of-the-art radiation sources and instrumentation. RADCAL will extend our research capabilities in personnel dosimetry and complement the program at the Health Physics Research Reactor.

We have a well-recognized nuclear medicine group that specializes in the development of new tissue-specific radiopharmaceuticals for medical diagnosis and therapy. Our work in this area extends to preclinical testing of new radiolabeled agents in laboratory animals and in vitro systems.



"ORNL West" in Grand Junction, Colorado, is staffed by 60 ORNL and subcontractor personnel who perform environmental measurements and assessments.

Clinical evaluations of our products are carried out by a network of medical collaborators at clinics and medical centers in the United States and Europe. The licensing of our advanced products for nuclear medicine applications is a goal of HASRD's technology transfer activities.



Steve Allman operates a laser to count krypton-81 atoms in a groundwater sample to determine whether the groundwater has been isolated or circulating.

Risk Analysis

Our work in epidemiology and risk analysis is an important element in our focus on human health. For example, our finding that hardness of drinking water (high levels of calcium and magnesium) seems to be linked to increased cardiovascular fitness in certain populations has attracted national attention. Our estimation and evaluation of health risks from radiation and chemicals have been continuing major efforts in HASRD. Some of this work has been done by the division's Office of Risk Analysis, whose coordinator is editor-in-chief of *Risk Analysis: An International Journal*. Quantification of chemical risks is especially challenging because of the diversity of toxic chemical agents and the complexity of exposure situations. We have made several contributions to improving methodologies for estimating chemical risks to human health.

In October 1987, the Information Research and Analysis (IR&A) Section was transferred to HASRD, broadening our capabilities and giving us responsibility for its large computerized data bases of health- and energy-related information. These resources, which are shared with users nationwide, are used by the IR&A staff to evaluate and analyze data in special reports for the National Library of Medicine, the Department of Defense, EPA, and DOE.

HASRD's strong ties to educational institutions include part-time university faculty appointments of staff members, part-time and sabbatical research appointments in HASRD for university faculty, and research opportunities for graduate students. More than 200 Ph.D. and M.S. theses have been based on research in our division. The academic ties with students, faculty, and other guests have given us fresh ideas and sharpened the skills of our research staff. In turn, we have contributed substantially to training and educating new scientists.

In reviewing HASRD's accomplishments for the past 12 years, we see a long list of journal and book publications, I•R 100 awards (eight received since 1980), patents, product licensing agreements for technology transfer, and spin-off companies. We expect this level of achievement to continue. ornl

Stephen V. Kaye, who joined ORNL's staff in 1960, has been director of the Health and Safety Research Division since 1977. He received his Ph.D. degree from the University of Rochester Medical School. He is a fellow of the American Association for the Advancement of Science and coeditor of the book *Indoor Air and Human Health*.

40 Years of Radiological Physics Research

Research on the interactions of radiation with matter provides the basis for sensitive detectors and better health protection.

By G. Samuel Hurst

I have long thought that the appropriate physics perspective for understanding biological phenomena is at the atomic or molecular level. Of course, a more fundamental level exists. The atom is made up of particles (protons, neutrons, and electrons) and, according to particle physicists, the nucleons (protons and neutrons) are made up of quarks, which, presumably, along with other entities called leptons, are *the* fundamental particles. However, in considering the study of life (biology), I think the ancient Greeks had the right idea. They held that the "atomos" is the indivisible particle that is conserved when a plant or an animal progresses through its life cycle, finally returning atoms to the eternal "atom stream."

For many years, the interaction of radiation (e.g., gamma rays, sunlight) with matter (e.g., water, living cells) has been an important focus of the physics programs in the Health Physics Division and, later, the Health and Safety Research Division at ORNL. One of the purposes of this radiation physics research was to understand the interactions of radiation with atomic and molecular systems (including gas, liquid, and solid-state systems) to obtain meaningful information for radiation chemistry and radiation biology. On the practical side, the physicists were responsible for applying essential physics information to the development of new instruments for radiation protection in the workplace. Because of this duality of purpose, physics research occupied a prominent place in ORNL's health physics programs.

With these two closely related objectives in mind, Karl Z. Morgan, then director of the Health Physics Division, initiated a basic program in radiation physics in the early 1950s. Our studies of radiation interactions with solids have led to fundamentally new concepts in physics, recognized worldwide for their beauty as well as relevance (see Ritchie's article on p. 162).

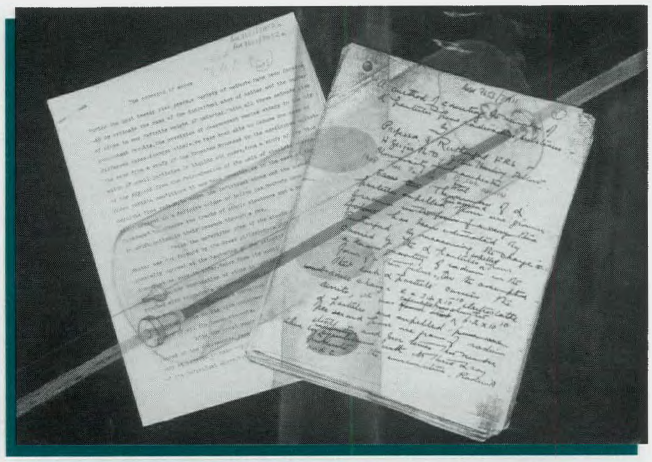
Radiation Protection Studies

My interest in radiation physics started in the company of theoreticians Rufus Ritchie, Jake Neufeld, and Walter Snyder. In a meeting with Morgan and these theoreticians, it was suggested that T. E. Bortner and I set up some new experiments to measure the total ionization produced when an alpha particle dissipates its energy in a gas. These simple experiments provided data needed to calibrate the ionization detectors used for radiation protection.

As often happens, simple experiments require elaborate speculations, leading to new theories and to far more complex experiments. Thus, we set up a highly integrated but varied experimental and theoretical program to attempt to understand nature's simplest example of the interaction of radiation with matter—swift protons striking a helium atom. One effect particularly attracted our attention. It was observed as early as 1915 that very small quantities of impurities in helium gas could

Sam Hurst conceived the idea of single-atom detection using resonance ionization spectroscopy.

Ernest Rutherford and Hans Geiger, who invented the proportional counter in 1908, wrote about the possibility of counting individual atoms. The laser (superimposed on their writings) made it possible.



cause the total ionization to increase by about 50%. Niels Bohr commented on this surprising effect, later known as the Jesse effect because of the dedicated work of W. P. Jesse at Argonne National Laboratory in the 1950s. This approach taught me that the pursuit of a simple problem often opens up a wider horizon. We were successful not only in obtaining badly needed practical data but also in understanding this effect at the level of the atom and electron.

The radiation physics and dosimetry program grew to about 25 permanent staff members in the early 1960s, plus an average of about 10 graduate students, primarily from the University of Tennessee and Vanderbilt University. By this time, we were studying the ability of matter to stop penetrating radiation (stopping power), excitation and ionization processes, the motion of electrons in gases, the capture of electrons to make heavy negative ions, and ion-molecule interactions. We also had major theoretical efforts relating to the transport of radiation in matter.

Former ORNL Director Alvin Weinberg has noted the strong interaction between basic and applied research and how they strengthen each other. Our basic research results on total ionization and ionization interactions in the gas phase cleared up some long-standing puzzles in radiation physics and helped radiation chemists better understand their experimental results. In turn, both radiation physics and radiation chemistry were essential for grasping the principles of radiation biology.

On the practical side, our applied research data on electron mobility, attachment, and diffusion improved our understanding and design of detectors for radiation dosimetry. I recall with considerable nostalgia the excitement experienced by the dosimetry group (E. B. Wagner, W. A. Mills, and others) when we developed several kinds of dosimeters that could actually measure the energy absorbed in living tissue. These devices were based on the proportional counter, invented by Ernest Rutherford and Hans Geiger at Manchester in 1908.

The ORNL group used proportional counters that measure fast neutrons and ignore the gamma radiation that is less effective in producing biological damage. The use of these dosimeters was vital here and around the world in measuring the hazardous neutron radiation produced by reactors and other fission sources. A different class of detectors was required to measure neutrons from atomic weapons. "Threshold detectors" (containing small samples of materials that would fission as a result of neutron bombardment) were quickly developed in response to an Atomic Energy Commission request that we perform neutron dosimetry for weapons tests in Nevada.

Resonance Ionization Spectroscopy

When the AEC charter was changed in 1975 to include other forms of energy (i.e., the transition from AEC to DOE), we were asked to broaden our research outlook. This challenging task was easier for us than others because we had been working at a basic level. For example, our studies on electron transport and negative ions were readily adaptable to the problem of determining health and environmental effects of chemicals released to the atmosphere. Our research on energy pathways following radiation absorption in gases led to the development at ORNL of resonance ionization spectroscopy (RIS), an important analytical chemistry tool for counting ordinary atoms. The ability of proportional counters to detect even a single electron gave me the idea of using lasers for single-atom detection. I am grateful to several colleagues who formed a team with me to carry out this exciting work.

Basic research is a remarkable pursuit, and those who take part in it are, indeed, privileged. I recall once boasting that basic research could be justified to anyone and that studies on electron transport in gases could be sold to the fire department. Although that certainly sounded daring at the time, look at the now-common smoke detector. To my chagrin, we should have, but did not, invent this device! In our research programs, we studied three phenomena (ionization of gases with alpha particles, electron mobility, and electron capture) that have been used in various versions of the smoke detector.

Two important programs grew out of our research on electron interactions and the transport of electrons through gases. As a graduate student, Bob Compton studied time-of-flight mass spectroscopy of negative ions to better understand the fate of electrons in a gas. His work blossomed to include photodetachment and photoionization studies and has led to new ion sources for particle accelerators. Loucas Christophorou continued with electron swarm studies, which were broadened into a variety of basic studies on large molecules. On the practical side, the Christophorou group has pioneered the field of gaseous dielectrics—so important to the electrical power industry.

One of the most important reasons for so much success in radiological physics at ORNL is the strong interplay here between theory and experimentation. In all of my early work with radiation detectors and dosimeters, I had the good fortune to team with Ritchie, Neufeld, Snyder, Ray Garrett, and Harvel Wright. These theorists were available for consultations on the practical radiation dosimeters and were especially helpful in suggesting basic experiments that would build a base for future innovations. Starting in 1949, I worked closely with Ritchie in developing neutron dosimetry. As a colleague, he taught me that a knowledge of theory and an ability to make quick calculations can save considerable time in experimental work. Also, James E. Turner and I collaborated on a book on elementary radiation physics. Since the 1970s, I have had the good fortune of working with Marvin Payne on the interaction of charged particles with matter and the use of laser techniques to study these interactions. This close collaboration led to the development of RIS for single-atom detection and a book he and I wrote on this subject.

Technology Transfer

In the currently emphasized area of high-technology inventions and product innovations, a number of commercial products have come from the 40 years of radiological physics at ORNL. These include the licensing of three separate neutron dosimetry technologies (to Raychronix, Reuter Stokes, and EG&G) and the formation of several new companies (Elographics, Atom Sciences, and Comstock) for the development and marketing of other products. My opportunity to be a co-inventor of the "touch screen" (developed by Elographics) was a thrilling experience. Ernest O. Wollan, who was one of ORNL's most respected physicists and who, with Morgan and others, pioneered the field of health physics at the University of Chicago, advised me that physicists can also be inventors and through invention can participate—even as national laboratory researchers—in free enterprise, a cherished cornerstone of American democracy. How fortunate we and our society are today that there is much wider recognition of this possibility. ornl

Sam Hurst, a former section head in ORNL's Health and Safety Research Division, helped develop neutron dosimetry, resonance ionization spectroscopy, and single-atom detection and started three new companies—Elographics and Atom Sciences, Inc., in Oak Ridge and Consultec Scientific Inc., in west Knoxville—and a Laser Technology Center to conduct applied research and provide laser training.

Fundamental Interactions in Condensed Matter

By R. H. Ritchie

The challenges facing physicists working in our division's interdisciplinary environment are many, varied, and always interesting. It has been my good fortune and pleasure to work with many highly talented and creative people at ORNL and elsewhere. When I joined ORNL's Health Physics Division (now the Health and Safety Research Division) in the fall of 1949, a few physicists were working on various problems in measuring personnel exposures to radiation. Among these were Francis Davis, Paul Reinhardt, Joe Cheka, Jake Neufeld, and Sam Hurst. Karl Z. Morgan, then division director, strongly encouraged both basic and applied research related to radiation dosimetry. Under the leadership of Morgan and subsequent directors John Auxier and Steve Kaye, the staff has grown from this small nucleus to two sections of about 35 full-time researchers engaged in a wide variety of basic physical studies on the interaction of radiation with matter.

It was very stimulating to work with Sam Hurst during the early part of his career, when he was pioneering new concepts in radiation dosimetry, and later, when he established the Japanese Dosimetry Program (to determine radioactivity doses received by Japanese populations from the atomic bombs dropped on Hiroshima and Nagasaki) and techniques for nuclear accident dosimetry.

Another outstanding colleague, Bob Birkhoff, joined the Health Physics Division as a consultant from the University of Tennessee (UT) in the early 1950s. Birkhoff built a beta-ray spectrometer that measured energy losses of swift electrons after penetrating thin foils. The idea was that such physical data would help us understand radiation interactions in solids and liquids (condensed matter) of biological interest. It was known even then that the spectrum of valence electron excitations in solids can differ markedly from that in the isolated constituent atoms.

Plasmons

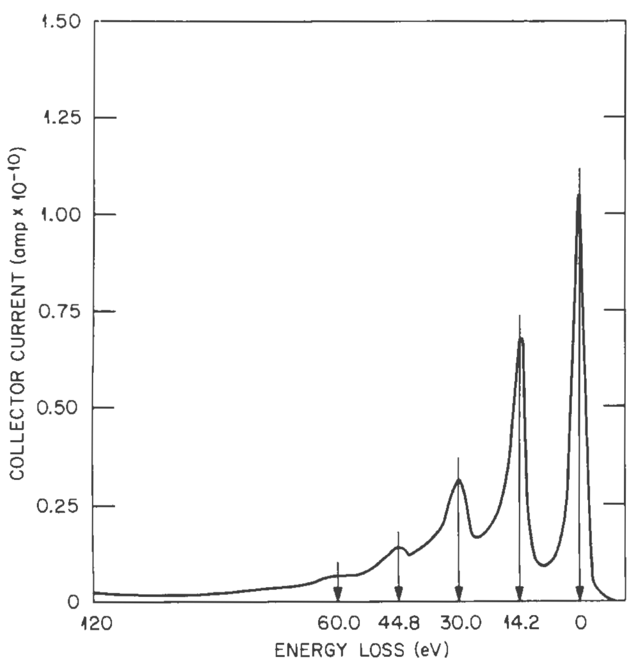
The word plasma usually evokes a picture of a hot, turbulent, and completely ionized state of matter. Like the gaseous discharges in tubes built by Irving Langmuir in the 1930s, a plasma suggests a host of free electrons moving rapidly amid a background of dynamic, positively charged ions. Curiously, a simple piece of metal, though relatively stable and cold, also qualifies as a "plasma" in the view of modern physics. In this case, the plasma has a fixed background of positive ions and a host of electrons free to move throughout the volume, as indicated by the high electrical conductivity of the metal.

If the time-varying electric field of a moving ion or electron acts on a piece of metal, the field polarizes the sample to form a time-varying electric charge density. The induced charges act on one another in a collective manner to try to restore equilibrium. If the applied field is removed, the rush toward equilibrium gives the mobile charges more kinetic energy, causing them to overshoot the equilibrium point. The process subsequently reverses and plasma oscillations are established.

The wave-particle duality of quantum mechanics assigns particle-like properties to waves as well as wavelike properties to particles such as electrons. The quanta associated with waves propagating in matter through the collective motion of large numbers of electrons are called plasmons.

In the 1950s, I was working part-time with Jake Neufeld, a wise and far-seeing theorist, on some aspects of nonlocal dielectric theory, which deals with dynamical, long-range effects of solids on penetrating charged particles. We developed a dielectric formulation of the response of a quantum plasma and studied the spatial and temporal distribution of excited electrons about a swift ion in such a medium. This theory has had recent important ramifications in understanding "channeling" of ions through open regions in crystals. About that same time, David Pines and David Bohm, then of Princeton University, predicted that metals should exhibit quantal excitations, or plasmons, corresponding to the oscillations that were known to exist in classical plasmas.

Birkhoff and I surmised that certain measured characteristic peaks in the energy-loss spectrum of electrons penetrating metal foils might result from plasmon generation. We analyzed spectra of these losses, assuming that each given electron could create many such plasmons if the foil were thick



Electrons penetrating a thin aluminum foil tend to lose energy in discrete units. Most pass through with no effect, accounting for the large peak at zero energy loss. Others excite electrons in the aluminum, which oscillate collectively, but only at the quantized energy levels indicated by successive peaks in the graph.

enough, and found that a Poisson distribution fitted the data well and that theory gave good agreement with the measured mean free paths for plasmon excitation by the incident electrons. Electrons penetrating a thin metal foil tend to lose energy in discrete units. Most pass through with no effect, accounting for the large peak at zero energy loss. Others excite free electrons in the aluminum, which oscillate collectively, but only at the energy values given under successive peaks in the graph. The exchange of energy between bombarding electrons and electrons in the metal is now explained by considering the metal as a plasma and the electrical oscillations as particle-like quanta called plasmons. Our data, plotted in the figure shown here, were the first measurements and analyses of such losses in terms of the plasmon model.

I became curious about how the energy-loss spectrum might be affected by the presence of boundaries (e.g., solid surfaces) and worked out the theoretical dynamics of the coupled fast electron–thin foil system. It turned out that completely new features appeared in the excitation spectrum of the bounded electron gas (electrons confined to the solid) as a result of the coupling of the polarization charges appearing at its surfaces. The polarization is caused by the penetrating electrons, which attract positively charged particles and repel negatively charged particles in the solid. The characteristic frequency of the oscillating electrons at the surface of a thick system is smaller than the volume plasma frequency because of the depolarizing effect of the boundary. The quantum of this oscillation is called the surface plasmon (SP).

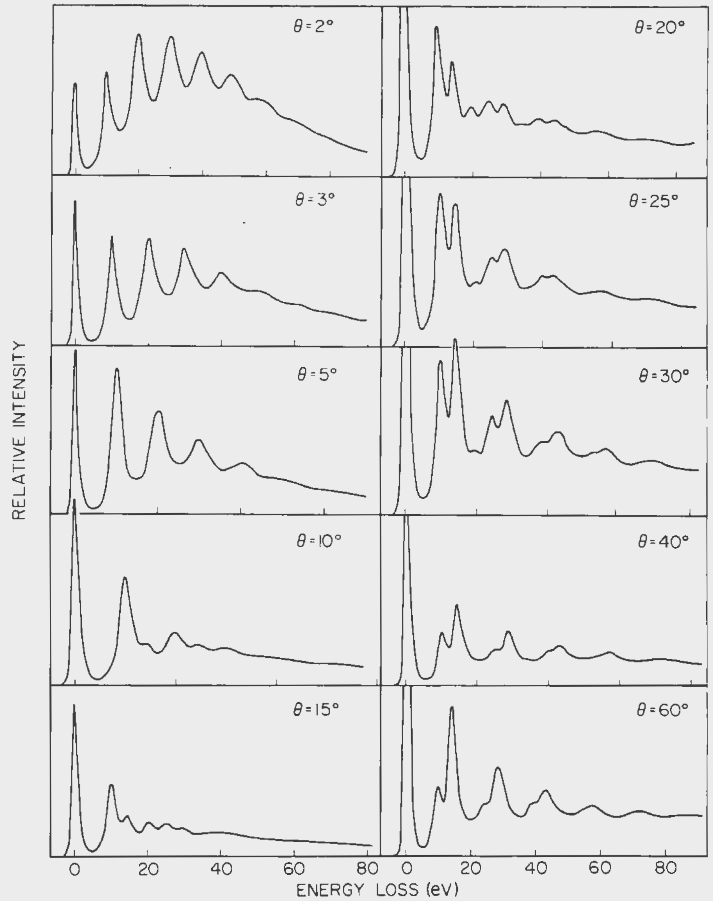
Surface Plasmon Prediction

Although I could find no definitive evidence in the literature that electron energy losses to SPs had been observed, I believed there was an appreciable probability of finding SP generation in a properly designed experiment. So, I described the distinguishing characteristics of losses to these modes and published the paper in *The Physical Review*.

About three years later, Cedric Powell and John Swan of the University of Western Australia confirmed experimentally the existence of SP losses by electrons. The SP has proved to be of great importance in the rapidly developing field of surface science. It is a prominent feature in electron energy loss experiments, low-energy electron diffraction, Auger electron spectroscopy, photo-emission experiments, catalysis, surface-enhanced Raman spectroscopy, and many other areas of research.

In recent years SPs have been shown to produce many striking optical effects and are being investigated for potential applications in holography, solar cells, miniature light sources, new types of microscopy, ultrasensitive chemical detection instruments, submicrometer physics, and other areas. The SP has been shown to be intimately involved in the "image force" in which a charge outside a solid surface elicits an equal and opposite charge inside the solid at the same distance from the surface. A high degree of current interest in the SP is illustrated by the designation of my original paper on the subject as a "Citation Classic" by *Current Contents*.

Birkhoff and I worked closely in analyzing a set of measurements that he and his co-workers made on the spectra of electrons slowed down in solids. This set of measurements, plotted in the figure on this page, remains the only such data even today.



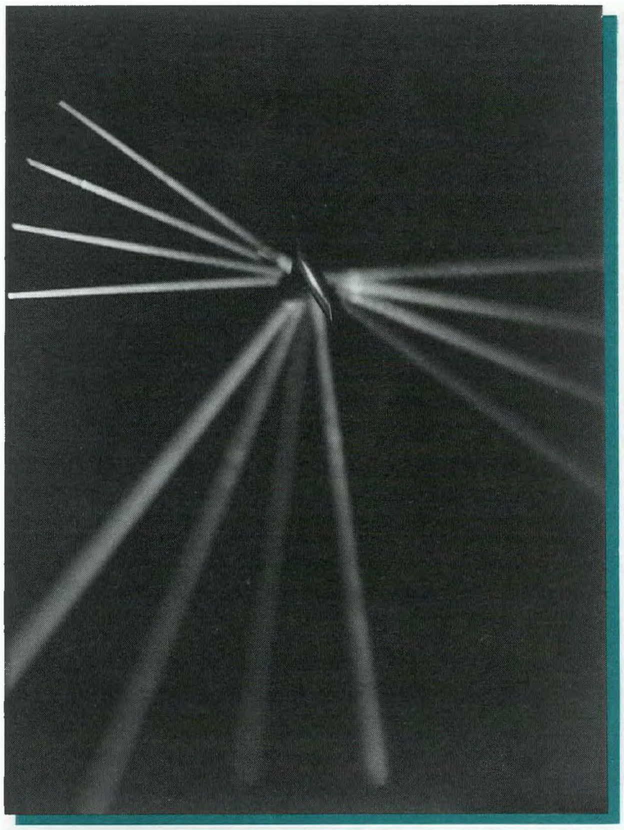
A fast electron beam excites two types of plasmons (quantized electron oscillations) as it reflects from a metal surface. At low angles of incidence, the plasmons are confined to the surface; as the angle of incidence increases (top to bottom and left to right in the figure here), the oscillations spread throughout the volume, and bulk plasmons, with higher and more distinct energy peaks, predominate.

A fast electron beam reflected from a metal surface excites two types of plasmons. At low angles of incidence, near grazing (top), the beam produces surface plasmons in the metal (oscillations of electrons confined to the surface). As the angle of incidence increases (top to bottom and left to right in the energy-loss plots of the figure), the oscillations spread throughout the volume, and bulk plasmons begin to dominate. Since these have slightly higher energies than the surface plasmons, their peaks become more and more distinct.

We made several theoretical constructions of spectra in different solids for comparison. This effort led us to develop new approximations for the dynamic response functions of solids (e.g., response to perturbations by charges) that have been very useful in arriving at parameters related to the transport of electrons through solids. This work is the basis of continuing research in our group.

Theory Developments

To better understand how surface plasmons are manifested in experiment, we subsequently developed the theory of light emission from solids bombarded by swift charged particles, accounting for both surface plasmon–photon coupling and the generation of bremsstrahlung, or "braking radiation" emitted when a penetrating particle is "slowed down" in the solid. This theory was



Reflection of four incident beams from a quartz slide coated with microparticulates of silver. Excited plasmons on the silver surfaces give color to the reflected light (shown here as shades of gray).

developed in close conjunction with the experimental work of Ed Arakawa, who also joined the Health Physics Division in the early 1960s and whose outstanding ingenuity and expertise in the laboratory have enabled him to make many important contributions to surface physics. We derived the first mathematical expression for the characteristic energy of the fully retarded SP during this period. This work has significant implications in understanding surface electromagnetic waves in solids, which are currently being investigated intensively in areas such as integrated optics. We were the first to apply hydrodynamic theory and quantum dielectric theory to study the SP within the "specular reflection model," now used as a standard theoretical tool to study collective modes such as surface plasmons and surface polaritons.

During the 1970s, we worked on many aspects of plasmon physics, including the properties of such excitations in spheres, the Wood's anomaly in diffraction gratings, the probability of double-plasmon emission in electron energy-loss spectroscopy experiments, the effect of exchange on the inelastic mean free path of a low-energy electron in a metal, the properties of electron-hole cascades in an electron gas, and the anomalous damping of plasmons in polycrystalline metals.

In collaboration with Archie Howie of Cavendish Laboratory in England, we addressed problems of electron microscopy. For example, we devised a criterion for the maximum resolution attainable in energy-loss transmission electron microscopy and scanning microscopy and developed a statistical theory of the optical potential appropriate to such work. In a subsequent collaboration, we proved a general theorem about energy-loss spectroscopy using electron microprobes. This work is relevant to understanding the spatial distribution of energy deposition in condensed matter and, thus, has important implications for microdosimetry.

Also during the 1970s, we became involved with new aspects of the theory related to stopping swift particles in matter by carrying out the first nonlinear analysis of the physics of the process, elucidating the characteristics of the space-time disturbances created, and studying the interaction of ion clusters in condensed matter. This laid the groundwork for a new experimental method of establishing the steric (geometric) structure of ion clusters.

Researchers important to the continuing development of our programs joined our staff in the early 1960s. Among these were Harvel Wright, now head of the Biological and Radiation Physics Section, who helped pioneer important theoretical studies of the transport of electrons in liquid water using Monte Carlo methods. Other talented researchers involved in this work include Bob Hamm, formerly a UT graduate student, and Jim Turner, who came to us from the Atomic Energy Commission and was recently named a Corporate Fellow of Martin Marietta Energy Systems, Inc. This group, together with Neufeld, used the Monte Carlo approach over a long period to study the transport of pions and other high-energy radiation for dosimetry applications. Jim Ashley also joined our division in the 1960s and has made important contributions to our basic work in understanding radiation interactions with atoms in solids.

Monte Carlo Techniques

An important dosimetry development at the microscopic level (microdosimetry) has been the use of Monte Carlo techniques to simulate the time and spatial evolution of charged-particle tracks in matter. A computer program developed by Bob Hamm, Jim Turner, and Harvel Wright to accomplish this is described more fully in the article starting on p. 182. We have devised theoretical models for predicting collision cross sections over a wide range of energies for electrons and ions in liquid water, using optical data obtained by Birkhoff and Linda Painter. Cross sections of optically active, as well as acoustic, modes of the liquid have been modeled. This work contributed to the first realistic theoretical microdosimetry research.

Recent Research Directions

When the federal government changed its emphasis in the 1980s to include broader aspects of energy production and control, we were asked to shift our research directions correspondingly, building on our basic understanding of condensed-matter interactions. We began studying the physics underlying basic aerosol interactions, including frictional forces on an atom near a surface, ripplon effects (the interactions of atoms with quantized surface waves on liquids), and aspects of physisorption (the adsorption of atoms on surfaces by van der Waals forces). We devised a new and powerful technique for treating the self-energy of atoms or charged particles (energy of interaction with the polarization they create) and applied it to analyze quantal effects in a number of important systems. Other gifted researchers who joined our section during the last decade, such as Oakley Crawford, Tom Ferrell, and Bruce Warmack, have added much to our programs.

Among the many excellent aspects of working at ORNL is the possibility of carrying out collaborative research with colleagues at other institutions. I was fortunate to have had several such assignments. These were extremely stimulating and fruitful, allowing developments of new techniques and concepts through contact with active and talented people such as Jens Lindhard (Aarhus University, Denmark), Archie Howie (Cavendish Laboratory, England), and Pedro Echenique (University of the Basque Country, Spain). Other outstanding researchers with whom I have collaborated include the late Werner Brandt of New York University, Dick Manson of Clemson University, C. C. Sung of the University of Alabama at Huntsville, Alberto Gras Marti of the University of Alicante, Spain, and Fernando Flores of Autonomous University of Madrid, Spain. I owe much to the inspiration and stimulus of these fine individuals and, especially, to the support and encouragement of Hurst, Morgan, Birkhoff, and Neufeld over the years.

As we move toward the 1990s, it is clear that fundamental physics research has made, and will make, many contributions to an increased understanding of radiation effects in condensed matter. In the words of our former colleague, Werner Brandt, in whose memory we dedicate an annual Workshop on Charged Particle Penetration Phenomena, "Supporting basic research is like stockpiling gold in Fort Knox." ornl

Rufus Ritchie, who came to ORNL in 1949, is a theoretical physicist in ORNL's Health and Safety Research Division. He has a Ph.D. degree from the University of Tennessee, has received fellowships from Churchill College and Cavendish Laboratory (both in England), and has been a visiting professor at Odense and Aarhus universities in Denmark. He is a Corporate Fellow of Martin Marietta Energy Systems, Inc., a fellow of the American Physical Society, and a recipient of the Jesse W. Beams Award for Excellence in Research from the Southeastern Section of the American Physical Society.

Ions, Molecules, and Energy

By R. N. Compton and Cornelius E. Klots

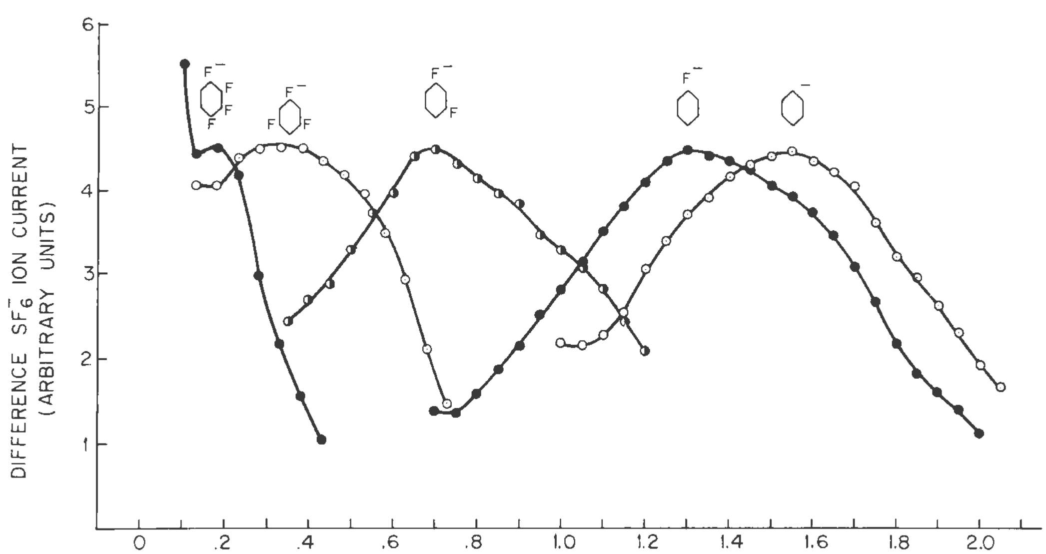
ORNL chemical physicists measure electron affinities, discover "super halides," study metastable negative ions, and pioneer in multiphoton ionization spectroscopy.

Chemical physics is, as the name suggests, a branch of science that sits astride the interface between physics and chemistry. Some might identify it with physical chemistry, but there are some subtle distinctions. A physical chemist might be more interested in the acidity of a glass of water, whereas a chemical physicist would more likely ask whether acidity has any meaning in a cluster of, for example, 50 water molecules. A gathering of chemical physicists would probably contain equal numbers of chemists and physicists.

For the past several years, the Chemical Physics Section in ORNL's Health and Safety Research Division has obeyed this rule of thumb. Its roots lie in early studies of radiation chemistry and radiation physics and in the development of new radiation detectors. These activities continue today, along with added responsibilities in atmospheric chemistry and pollutant detection.

Initially, we were interested in the physical and chemical changes that occur in matter subjected to ionizing radiation. The physics part of the approach addressed energy deposition questions—for example, when an alpha particle is stopped in matter, where does the kinetic energy go? We know that initially this energy is apportioned among the many primary and secondary electrons produced. The secondary slow electrons create a multitude of excited species and free radicals. These reactive species induce the chemical changes in matter that constitute radiation damage. Thus, an interesting and important part of the interaction of ionizing radiation with matter lies in the fate of the numerous slow electrons.

Many early studies in radiation chemistry made use of the sulfur hexafluoride (SF_6) molecule. Two characteristics of SF_6 were especially interesting. First, it has the intriguing property of attaching only electrons of very nearly zero energy. Neutral molecules of SF_6 deliberately added to a medium having such slow electrons easily form negative ions, and so can be used to detect these electrons. Second, an isolated SF_6^- ion may re-emit its excess electron and return to a neutral state. The rate of this emission is slow, partly because the SF_6 constitutes a deep trap for electrons. Our own studies have gone far beyond the SF_6 molecule. Nevertheless, its properties will serve as a common denominator for the discussions that follow.



Using the SF_6^- scavenger technique, ORNL scientists discovered these "negative-ion resonances" for a series of fluorinated benzenes.

Negative-Ion Resonances

Not all molecules hang on to an electron as long as SF_6. It is much more common for an electron to form a negative ion having only a fleeting existence. In the early 1960s, George Schulz at the Westinghouse Research Laboratories discovered such "negative-ion resonances" in helium and nitrogen and found that these occur only when the energy of the electron exactly matches an energy gap in the negative ion. The negative-ion resonances in nitrogen (N_2) and carbon dioxide (CO_2) have made possible the CO_2 laser, for example. Infrared light from this laser results from the stimulated emission of vibrationally excited CO_2 molecules, which are produced by electron impact on CO_2 molecules and also by vibrationally excited N_2 molecules. In either case the excitation is a result of resonances.

Shortly after Schulz's announcements, we discovered resonances in a large number of organic molecules, using the "SF_6 scavenger" technique. In this approach, electrons emitted by electronically or vibrationally excited negative ions are picked up, or scavenged, by nearby SF_6 molecules. By detecting the newly formed SF_6^- ions in a mass spectrometer, we obtained evidence for the brief existence of negative-ion resonances in benzene and other organic molecules. The figure on the preceding page shows the now well-studied ~1.5-eV resonance in benzene detected by this technique and the effect that fluorine substitution in benzene has on the position of the negative ion resonance.

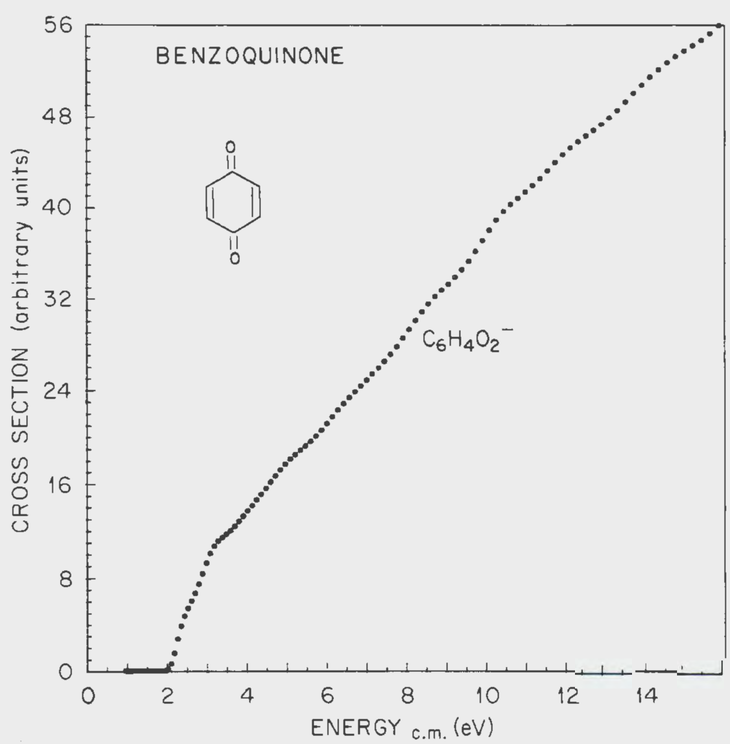
Measuring Electron Affinities

The electron affinity of an atom or molecule is the "binding" energy for an extra electron and may be either a positive or negative value. In the case of negative-ion resonances, the affinity for electrons is "negative" because the molecule takes on energy when it picks up an electron—that is, energy from outside the molecule is required to attach the electron. Conversely, an atom or molecule that has a "positive" electron affinity gives up energy upon attaching an electron. Determining electron affinities—whether positive or negative and how much energy is absorbed or released—is notoriously difficult, especially for molecules. We developed a novel charge-transfer method for measuring electron affinities of large molecules. Attesting to its timeliness, similar techniques were simultaneously developed by E. W. Rothe (Wayne State University) and Dudley Herschbach (Harvard).

Our method involves measuring the threshold for charge exchange from an alkali atom (A) to a molecule (M),

$$A + M \rightarrow A^+ + M^- .$$

The figure below shows the cross section for charge exchange between fast cesium atoms and *para*-benzoquinone (PBQ) as a function of



Cross section for charge exchange between fast cesium atoms and *para*-benzoquinone (PBQ) as a function of collision energy.

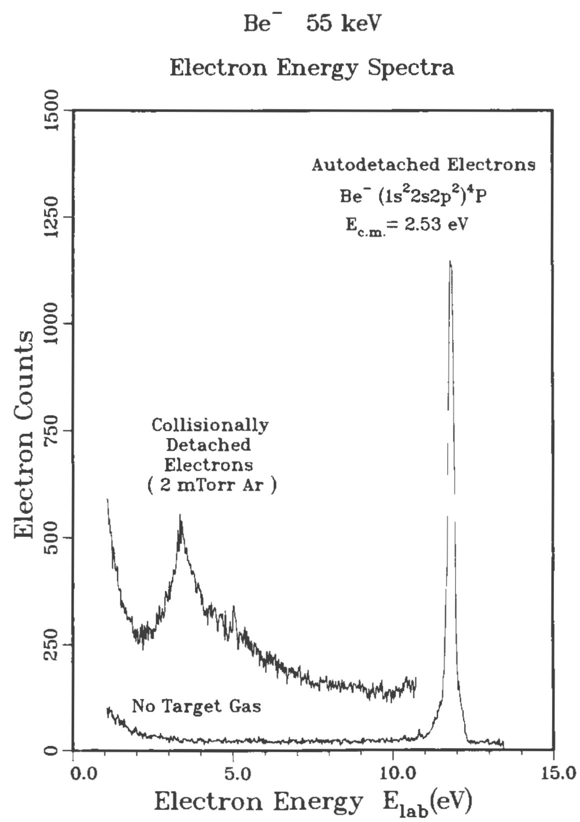
collision energy. The electron affinity of PBQ (1.89 eV) was deduced by subtracting the ion-pair threshold energy (2.1 eV) from the ionization potential of cesium (3.89 eV). The energy dependence of such cross sections (related to probabilities of charge exchange) above threshold are found to obey the Wigner threshold law—that is, to be very nearly a step function. (The law was discovered by Eugene Wigner, former ORNL director and Nobel laureate.)

Because the electronic structures of all organic molecules are similar, we were able to calculate the electron affinities of such species using a semi-empirical approach. J. M. Younkin, a graduate student from the University of Tennessee (UT), related the measured electron affinities to the calculated energies for unoccupied molecular orbitals (energy levels). The electron affinity was taken to be directly proportional to the energy level of the lowest unoccupied molecular orbital, and excellent correlation was obtained. Once this correlation was well-established, we used the method to calculate the electron affinities of more than 120 organic molecules.

During these studies, a class of molecules, including platinum hexafluoride and gold hexafluoride, was discovered in which charge exchange occurred all the way down to approximately zero collision energy, indicating that the electron affinity of these molecules was greater than the ionization potential of the alkali atom. An important example of these "superhalide" molecules is uranium hexafluoride, whose electron affinity is now believed to be greater than ~8 eV.

Metastable Negative Ions

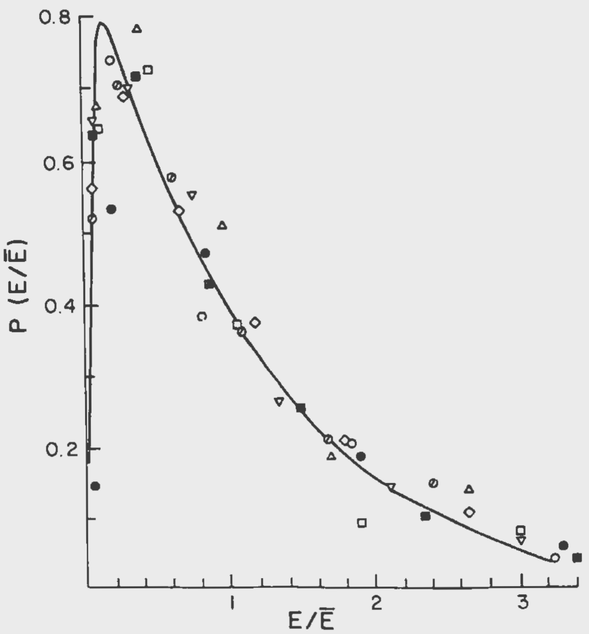
In 1982 Bob Compton began an important collaboration with Gerald Alton of the Physics Division, and later with Dave Pegg (UT Department of Physics), studying a special class of "metastable negative ions" in which an extra electron is bound to an atom or molecule already in an excited state. As this long-lived species relaxes back to its ground, or neutral, state, it ejects the weakly bound electron. One such example is the metastable negative ion of beryllium—$Be^-(1s^2\,2s\,2p^2)$, which is formed through a double charge exchange between a Be^+ ion and two separate alkali atoms—as when a fast Be^+ beam bombards a lithium target. In the figure below, the cusp-like feature at ~3.5 eV corresponds to collisionally detached electrons traveling at the ion-beam velocity. The peak at ~12 eV corresponds to autodetachment of electrons through the process $Be^-(1s^22s2p^2) \rightarrow Be(1s^22s^2) + e$.



Collisional and autodetachment electron spectra for metastable beryllium ions.

More recently, and mainly through the efforts of Pegg and Jeff Thompson (UT Department of Physics), we have determined the electron affinities of stable ions such as boron (B^-) and calcium (Ca^-) using fast-beam, laser-photodetachment electron spectroscopy. Before these studies, Ca^- was not believed to be stable (nor for that matter were any of the Group IIA metal negative ions). Our discovery that the negative calcium ion is stable was reported in 1988 by *Physical Review Letters*, several less-technical science magazines, and local newspapers.

A special type of negative ion, first studied at ORNL by Jim Turner and Ken Fox (UT Department of Physics), has only recently been experimentally verified. Some early experiments by Sam Hurst and John Stockdale suggested that polar molecules (those having opposite charges at opposite ends, or poles, of the molecule) might temporarily bind an extra electron because of their dipole moment (a measure of the molecule's charge separation). Turner and Fox confirmed that a nonrotating molecule whose dipole moment was greater than ~1.6 debye would bind an extra electron. Although Turner and Fox were the first of many to derive this result, they later found that Enrico Fermi and Edward Teller had, in passing, mentioned the value of 1.6 debye in a 1940 report



Kinetic energy spectrum for fragments from the dissociation of hot $C_3H_7I^+$ ions. The good agreement with theory identifies this spectrum as a reflection of the "reaction coordinate."

on electron-fixed dipole binding. ORNL's Ray Garrett and Oakley Crawford showed, however, that a rotating dipole (i.e., a real molecule) needs a dipole moment of ~2 debye to bind an additional electron. This and many other properties of polar negative ions and of electron scattering by polar molecules were theoretically described in the mid-1970s at ORNL. Some of these features have only recently been experimentally confirmed.

Chemical Dynamics

We mentioned earlier that an SF_6^- ion formed by direct electron attachment will slowly emit its electron. Calculating the rate of such a process shows that the usual chemical theories are not applicable. Eph Klots developed a more general way to address this dynamics problem. As a bonus, his approach also predicts the energy states of the final products from a given reaction.

Suppose an excited molecule has more than enough energy to dissociate, or break apart. Will this extra energy appear as kinetic energy of the emitted particle? Radiation chemists had frequently supposed that it would, but Klots predicted that the fragments would carry only a small fraction of the additional energy.

In the figure shown here, we see the kinetic energy distribution of fragments from the dissociation of propyl iodide ($C_3H_7I^+$) ions having a well-defined energy. Over most of the range, this distribution is a very nearly exponentially decreasing function of the energy. At very low energies, a depletion arising from centrifugal effects is also seen. The line through the data points represents Klots' a priori prediction.

The exponentially decreasing portion of the graph is a signature of an idea previously familiar to chemists only as a mental construct—the reaction coordinate. It is noteworthy that this construct can also be traced back to ideas of Eugene Wigner, formulated long before he came to Oak Ridge.

It is a long step from the single-molecule studies described above to an understanding of radiation effects in condensed media. Recognizing this, and noting also the increasing interest in energy-related contaminants in the atmosphere, we began directing our traditional techniques to investigations of small clusters of matter. Often called van der Waals molecules, these weakly bound aggregates of atoms or molecules can be generated in the laboratory with a sonic nozzle, an expansion device that chills gas molecules, sometimes reducing their vibrational and rotational energies enough for them to form molecular clusters.

As mentioned earlier, electron attachment to single molecules often occurs via brief "resonances"—that is, the molecules momentarily have an electronic energy level capable of accepting an extra electron. In clusters of increasing size, we saw these resonances shift and evolve toward "solvation bands" characteristic of a condensed phase; in effect, electrons seemed to be "dissolved" by the closely packed gas atoms. Sometimes we observed new resonances having no precedent at the single-molecule level.

Of particular interest were species such as the negative ion of CO_2, which is metastable—that is, the extra electron is spontaneously ejected after an ion lifetime of ~90 μs. Solvation with only one additional CO_2 or water molecule was found sufficient to change its electron affinity from a negative to a positive value—it could retain its excess electron and not return to the neutral state. Likewise, we found that ionization of a cluster could trigger a series of ion-molecule reactions whose occurrence could only be surmised at the single-molecule level.

One role of the medium surrounding a molecule is to dissipate energy. This is illustrated by a reaction studied much earlier by Sam Hurst. He found that an electron can attach itself to a single oxygen molecule, only to be quickly re-emitted unless a stabilizing collision with a "third body" intervenes. We found that slow electrons can be attached directly to a preexisting oxygen dimer, which, in effect, offers a built-in third body. We called this phenomenon "evaporative electron attachment." It is now understood to be the mechanism by which free electrons disappear from the ionosphere.

Having characterized evaporative electron attachment, we turned to the dynamics of evaporation per se. Drawing on previous experience with unimolecular reaction theory, Klots was able to arrive at several scaling laws applicable to evaporation. Up to now, they have been tested with experimental measurements on molecular clusters from sonic-nozzle expansions. Nevertheless, they should be equally applicable to the aggregates vaporized from surfaces by sputtering and laser ablation.

Even without an extra electron, van der Waals complexes are interesting. We have developed a formalism that allows us to readily evaluate their thermodynamic properties. For example, we have calculated the pH of steam, thus addressing the question posed earlier: Is acidity a meaningful concept in small clusters? Seemingly esoteric, this result is directly pertinent to work in ORNL's Chemistry Division related to the properties of high-pressure steam in nuclear reactors.

Multiphoton Ionization

The invention and development of the high-power tunable dye laser in the mid-1970s changed chemical physics research almost entirely. It became immediately evident that this revolutionary new light source could provide new insight to many fundamental aspects of radiation research and atmospheric physics. Using this new tool, A. D. Williamson (postdoctoral student from the California Institute of Technology) and Bob Compton initiated one of the original studies of multiphoton ionization (MPI) spectroscopy. We were one of the original groups to enter this exciting area of chemical physics.

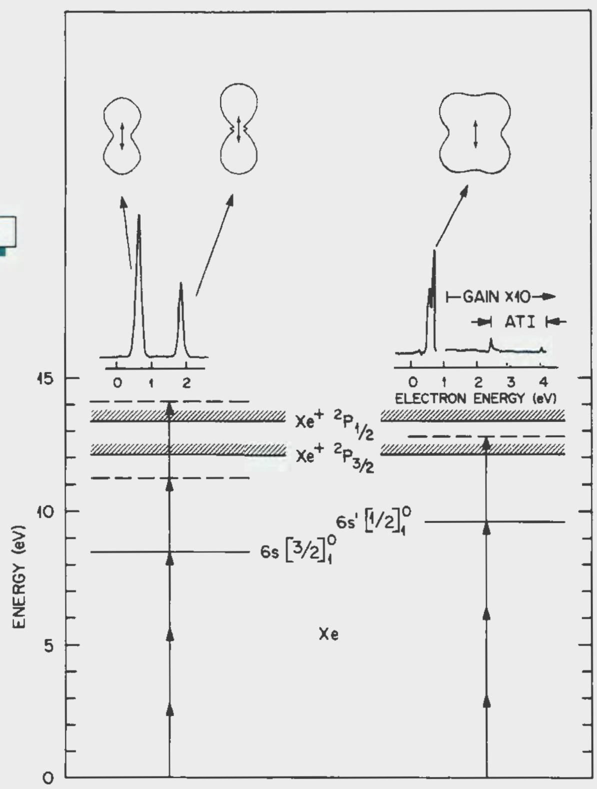
In addition to using the tunable dye lasers, John Miller and John Stockdale have been instrumental in developing frequency conversion techniques that extend the laser radiation into the vacuum ultraviolet region and further into the infrared region. These methods, which involve third-harmonic generation (a nonlinear optical effect in gases in which three photons of one energy are converted to one photon having three times that energy), wave mixing, and stimulated electronic Raman scattering, have played an important role in many areas of basic and applied research.

Early in our MPI studies, Miller and I observed that many three-photon resonances were not seen in the MPI spectra at modest pressures but were very intense at low pressure under single-collision conditions. Together with Ray Garrett and Marvin Payne, we later showed that three-photon resonances disappear because of the generation of third-harmonic radiation in the medium at low pressures. This disappearance occurs because the one-photon excitation and three-photon excitation are out of phase and cancel each other, resulting in *no* excitation.

Although not known to us, an analogous result was predicted by a group of Russian theorists in the mid-1960s. Our group has made subsequent contributions in both the theoretical and experimental description of this phenomenon. A physical model of the cancellation mechanism was provided by D. Jackson and J. J. Wynne (IBM) when they noted that the reflection of the laser beam upon itself restored the resonance-enhanced population at that level by thwarting the cancellation. This effect has now been observed in many atomic and molecular systems, and several unexpected consequences of the effect have been discovered here at ORNL.

Members of our group have been active in many MPI investigations, and we will mention only one other example. One-photon photoelectron spectroscopy (PES) of gas-phase atoms and molecules has provided unique information on the electronic, vibrational, and sometimes rotational states of gas-phase ions and added to our understanding of atoms and molecules. Miller and Compton have extended PES to multiphoton ionization studies (i.e., MPI-PES), achieving a marriage of two major technological advances in contemporary science: PES and the laser. The relatively new science of nonlinear optics provides the underlying physics of the processes involved. In this connection, it is worth noting that the 1981 Nobel Prize for Physics was shared by N. Bloembergen, A. Schawlow, and K. Siegbahn for developments in nonlinear optics, the laser, and PES, respectively. MPI-PES has become a field in itself and promises to surpass conventional PES in advancing our understanding of atoms and molecules. The figure on this page shows an MPI-PES spectrum of xenon atoms that also indicates the angular distributions of the photoelectrons with respect to the laser polarization direction.

In a current investigation, Miller and Howard Carman are applying MPI techniques and Rydberg atom-sensitization to the study of van der Waals molecules. The atom-sensitization is remarkably similar to the process of electron attachment and is, thus, another extension of our previous work. *Plus ça change, plus c'est la même.* ornl



Multiphoton ionization photoelectron spectrum of xenon atoms.

Robert N. Compton is a group leader in the Chemical Physics Section of the Health and Safety Research Division and a Corporate Fellow of Martin Marietta Energy Systems, Inc. He came to ORNL in 1965 after receiving a Ph.D. degree in physics from the University of Tennessee. He is also a Professor of Chemistry at the University of Tennessee.

Cornelius E. (Eph) Klots, a senior scientist in HASRD's Chemical Physics Section, came to ORNL in 1964 after obtaining his Ph.D. degree in physical chemistry from Harvard University. He is recipient of an annual HASRD Achievement Award and has been a Ford Foundation Professor of Physics at the University of Tennessee and a visiting professor at the University of Paris.

Resonance Ionization Spectroscopy

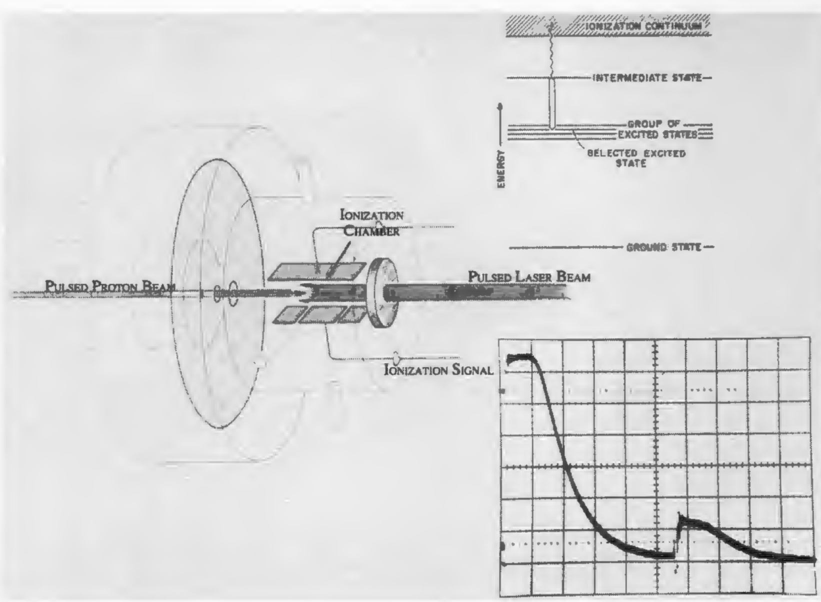
ORNL's chemical physics research leads to super-sensitive laser-based detectors.

By W. R. Garrett and M. G. Payne

While carrying out detailed studies of the excitations and subsequent relaxations (deexcitations) caused by fast charged particles moving through atomic and molecular gases, several ORNL physicists conducted a landmark experiment in 1975. Sam Hurst, Marvin Payne, Winston Chen, John Judish, and Munir Nayfeh performed an experiment that was to completely change the methodology for a large fraction of the experimental research in chemical physics at ORNL. That study led to the development of resonance ionization spectroscopy (RIS), an ultrasensitive technique for identifying and quantifying constituents at the atomic or molecular level.

As a charged particle (e.g., alpha particle) moves swiftly through a gaseous medium, its collisions with atoms and/or molecules leave a population of atoms in "metastable" excited states. Other atoms are left in "normal" excited states. These atoms can be detected, because they emit energy in the form of detectable light as they return to the "ground," or unexcited state. Metastable excited atoms cannot be detected optically, because they emit no light. However, because the atoms of interest are in an excited energy state, they should be distinguishable from all the unexcited atoms of the same type. Futhermore, because all atoms absorb light of very specific and quite distinguishable colors to move electrons between their individual energy levels ("resonant " wavelengths or frequencies), Hurst and his co-workers realized that a tunable laser could be used to resonantly excite the metastable atoms to even higher energies and then ionize them. They surmised that the electrons emitted during ionization would provide a detectable signal that would be uniquely associated with the species that had been excited by light corresponding to one of its resonant frequencies. In this way, the proportion of metastable atoms in a mixture could be determined without affecting any of the other atoms.

The first application of RIS was a rather exotic example, because the metastable helium atoms being measured were formed in relatively small numbers by protons from a pulsed van de Graaff accelerator (see figure). To carry out the selective ionization of these atoms, a tunable pulsed laser was used to produce photons of the unique wavelength needed to excite the electrons of the metastable helium atoms from their initial energy level to a higher excited state. A second photon from the same laser pulse further excited the electrons of the chosen atoms, removing them from the atoms completely and leaving helium ions. Since tunable dye lasers produce very intense light, the researchers showed that the process could be "saturated"—that is, the two-photon ionization process could be completed for every



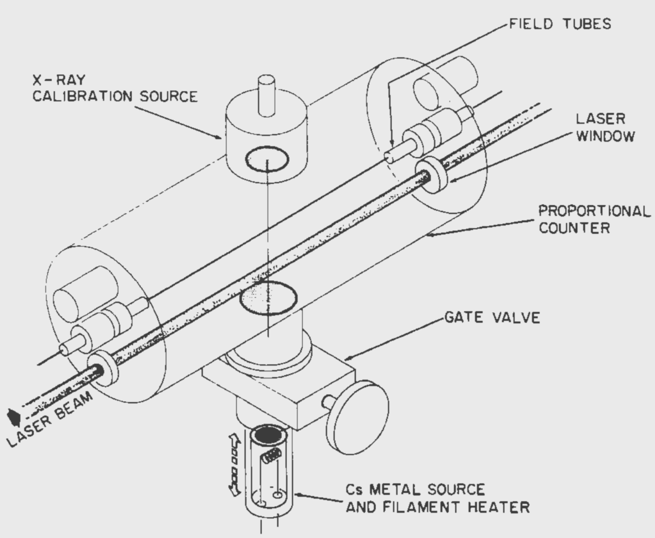
Schematic drawing of the first experiment involving resonance ionization spectroscopy (see text).

metastable helium atom within the laser beam. The sum total of these ionizations could then be measured as an electrical pulse whose magnitude reflected the total number of atoms ionized. In this demonstration experiment, the counter-propagating pulsed-laser beam resonantly excited and then ionized all the metastable atoms within the beam, and the pulse of electrons was measured. The ORNL researchers called their new process resonance ionization spectroscopy (RIS).

Single-Atom Detection

Our research team quickly realized the ultrasensitive detection capability that could be made possible by using a combination of tunable dye lasers to resonantly (and, therefore, selectively) excite and then ionize various chemical species, which could then be measured by charged particle detectors. Because even a single charged particle can be counted in a sensitive charge-detection device, this meant that even single ionized atoms could be counted within a laser beam.

One device capable of measuring a single ionization event is the gas proportional counter shown in the figure below. This device was used at ORNL by Hurst, Nayfeh, and Jack Young (of the Analytical Chemistry Division) in the first demonstration of single-atom detection, providing the basis for a very selective and sensitive analytical technique.



Laser and proportional counter system capable of detecting single cesium atoms.

In the initial study, a very low concentration of cesium atoms was introduced into the proportional counter, which also contained a low-pressure, inert, buffer gas. A pulsed laser was tuned to one of cesium's absorption frequencies and guided through the gas chamber of the proportional counter. The laser beam selectively ionized the cesium atoms that happened to be in the beam during a pulse. The electrons thus liberated (by RIS of cesium) were accelerated to a central charge-collection wire, creating an avalanche effect in the gas volume as the electrons rushed toward the wire and a resultant electrical pulse proportional to the number of electrons liberated (one from each cesium atom within the beam). Using the proportional counter apparatus shown in our illustration and compatible tunable laser, electronics, and data acquisition systems, they showed that single atoms of cesium could be detected and counted, even though they were present in a population of 10^{17} atoms and molecules of other types!

Containerless Chemistry

The ultrasensitive and highly selective properties of RIS opened many new research avenues in chemical physics at ORNL. Various phenomena have been studied using RIS, and an experimental ionization chamber was developed in which two or more laser beams passed through the internal gaseous medium (see figure on next page). The experimental chamber contained a low concentration of targeted molecules in a higher concentration of an inert gas. One laser beam was used to excite and dissociate (i.e., break apart) the molecules under investigation, while the second laser beam (or a pair of superimposed laser beams) detected and quantified the atomic fragments of a given type from the dissociated molecules. The dissociating laser was fired first, forming an initial population of dissociated atoms along the beam path. The second parallel laser beam (the RIS laser) was fired later and at a measured distance from the dissociated atoms. By changing the timing and/or distance of the second laser beam, the speed of atomic movement within the gas-filled cell could be measured, thereby determining the diffusion coefficient of that species.

If a reactive gas such as oxygen is present in the chamber, the atoms of interest that are excited by the first laser will react within the gaseous medium to form new molecules and thus will decrease in number with time. The resonance ionization measurement, which yields the number of excited atoms within the beam when the second (detector) laser is fired, can therefore be used to measure the reaction rate of the excited atoms with constituents of the reactive gas in which they are immersed.

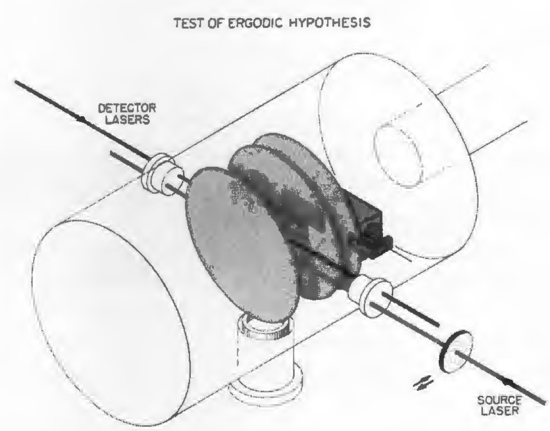
Finally, by spatially superimposing the two laser beams, all of the molecular dissociations that occur can be measured. By turning the laser power up high enough, all the molecules in a sample volume encompassed by the laser beam can be dissociated and counted, yielding the total molecular density without the use of a pressure gauge. The probability of dissociation per unit of laser power (called the dissociative absorption cross section) can thus be measured directly. These laser techniques allow us to study chemical reactions that happen very rapidly and in quick succession. Such research is sometimes called "containerless chemistry," because the reactive atoms liberated by the first laser never reach the container wall.

The RIS-based measurements described here help us to determine atomic and molecular properties relevant to atmospheric physics and chemistry and radiation physics. They may also lead to practical applications such as improvements in the designs of radiation and chemical detectors.

Resonance Ionization Mass Spectrometry

In the research described so far, no effort was made to determine the particular isotope of a given chemical species that might be present in a sample. Unless very special care is taken in the choice of lasers used in resonance ionization studies, all isotopes of an element will undergo laser ionization, because most lasers cannot be tuned finely enough to produce only the specific frequency needed to ionize a particular isotope. However, in many circumstances, we need to determine the isotopic, as well as chemical, identity of a given atomic or molecular species. We can do this by combining RIS with mass spectrometry.

In an ordinary mass spectrometric analysis of a sample, all constituents are ionized (by an electron current), and the relative abundances of all the various masses are recorded. However, because most atoms exist in more than one isotopic form, two or more constituents of a sample (i.e., isotopes of different elements) may have the same mass (isobars). Mass spectrometric analysis, which measures components only by mass, cannot differentiate these or provide the fractional concentrations of each species.



Schematic diagram of an experimental gas chamber used for "containerless chemistry" at ORNL.

However, by combining RIS with mass spectrometric analysis, each chemical constituent can be selectively ionized by its appropriate laser frequencies and these ions, which are all isotopes of that particular chemical species, can be subsequently quantified by the mass spectrometer. No confusion arises from the accidental presence of other constituents having the same mass, because no other species are ionized by those frequencies.

This combination of RIS and mass spectrometry is an extremely useful analytical chemistry tool. The work initiated at ORNL gave birth to a whole new analytical chemistry field, now designated as resonance ionization mass spectrometry (RIMS), which has many fascinating applications.

The Maxwell Demon: Dating Groundwater

Although laser ionization methods can detect even a single atom within a sample, and RIS coupled with mass spectrometry can provide an unambiguous isotopic analysis of the chemical species present, some problems are even more demanding—namely, the need to determine absolutely the total number of a very small population of atoms of a particular elemental isotope within a given sample. An important example in this category is krypton-81 (^{81}Kr), a radioactive isotope formed by cosmic ray interactions with stable Kr in the atmosphere.

The ^{81}Kr isotope has a half-life of 200,000 years, is chemically inert, and dissolves in water. Thus, it provides an excellent means for "dating" water, such as the groundwater in deep aquifers and water in polar ice caps. However, ^{81}Kr is produced at such low atmospheric concentrations that fresh water contains only about 1300 atoms of ^{81}Kr per liter. Very ancient water contains even fewer ^{81}Kr atoms, because many of them have undergone radioactive decay, the number depending on the age of the sample. The development of a useful ^{81}Kr-based technique for dating environmental samples required a capability for separating and counting about 1000 atoms of ^{81}Kr in the presence of 10^{15} atoms of ordinary Kr. This capability was first developed and implemented by Hurst, Payne, Chen, S. D. Kramer, S. L. Allman, R. C. Phillips, and Bernhard Lehmann (University of Bern, Switzerland) in a device described locally as the "Maxwell Demon."

An imaginary sorting demon, illustrated here, was introduced by J. C. Maxwell in 1872 and was described as capable of sorting atoms or molecules by allowing those of a certain type (or of a certain speed) to pass from one region to another (e.g., through a trapdoor) while closing off the passage to all others. The ^{81}Kr counting requirement is similar in character to the imaginary problems of Maxwell's sorting demon. Before we can count the atoms of a given isotope in a sample, the individual isotopes of the element must be sorted and stored until all have been inventoried. The device our ORNL group developed to accomplish this task is shown in the schematic drawing on the facing page. It incorporates RIS for selective Kr ionization, mass spectrometry for isotope (^{81}Kr) separation, and an electron "multiplier" detector for counting each Kr ion. However, additional features had to be added to this RIMS system to make it possible to count as few as 1000 atoms of ^{81}Kr in a small sample.

Depiction of J. C. Maxwell's imaginary "sorting demon," which could distinguish between types of atoms, sort them through a trapdoor, and store them until all have been separated and counted.

A few hundred noble gas atoms bouncing around inside an apparatus such as that pictured are extremely hard to count with a narrow pulsed-laser beam, because they seldom pass through the beam. Thus, an "atom buncher" was incorporated into the ORNL device to corral all of the Kr atoms into a small space. The buncher is actually a very cold (<10 K) finger probe, onto which all Kr atoms quickly freeze.

To begin RIS counting, a separate "evaporation" laser is fired to heat the cold finger and instantly evaporate the

Kr atoms directly into the RIS laser beams, where they are ionized. The Kr^+ ions are passed through a quadrupole mass-filter, which is tuned so that the ^{81}Kr isotope is transmitted 10,000 times more efficiently than any other isotope.

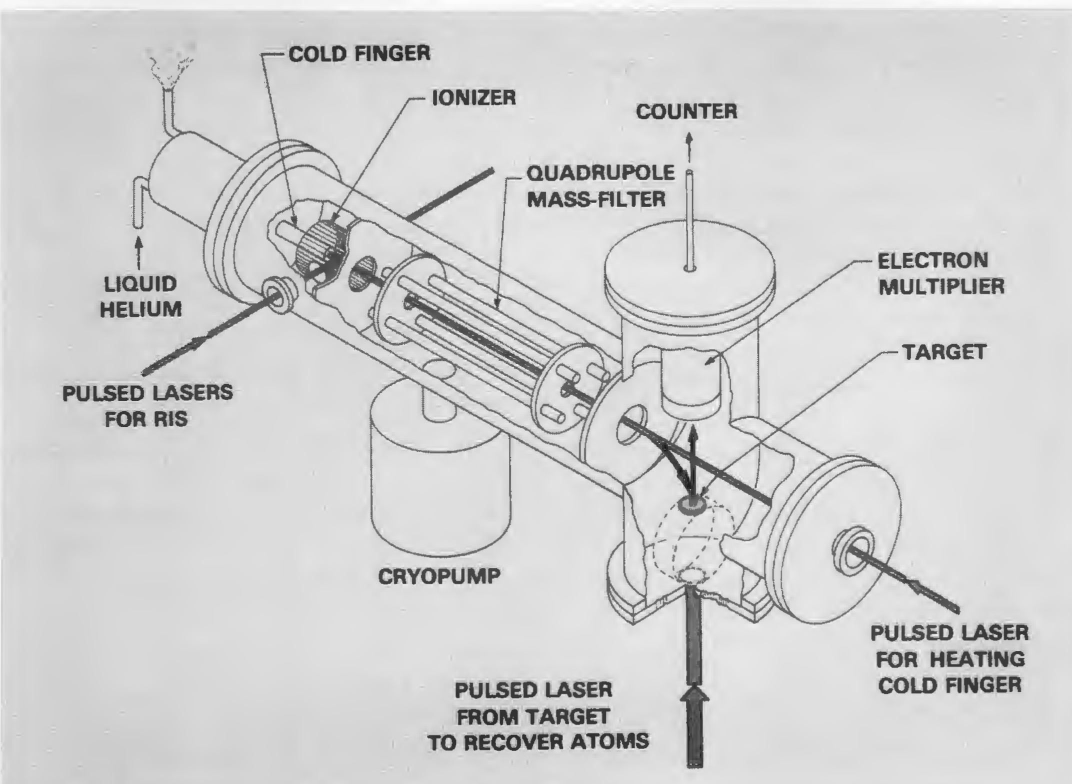
After being mass-filtered, the ^{81}Kr ions are embedded in a silicon target and, thus, stored. In the embedding process they can be counted, because several electrons are released and detected as the atoms enter the target storage disk. When all the selected isotopes are stored and counted, the remaining atoms can be "pumped" out.

In this step, all of the ^{81}Kr atoms and 10^{-4} of the original number of atoms of other Kr isotopes are separated, but the ^{81}Kr isotopes can be further "purified" by releasing the stored Kr atoms from the silicon trap and recycling through the counting and embedding procedure. This step is accomplished by adding a third (annealing) laser system that flash-heats the silicon atom trap, thereby releasing all the trapped Kr atoms. In the second cycle, only 10^{-8} of the original unwanted Kr isotopes are filtered through, allowing quite an accurate ^{81}Kr determination.

The laser-based Maxwell Demon isotope counter has been successfully used at ORNL to count ^{81}Kr atoms from groundwater samples. It is now being further developed by Atom Sciences, Inc., for dating polar ice caps and water from deep aquifers. Future environmental applications will undoubtedly include counting argon-39 atoms from seawater, because this radioactive isotope is useful for studying the mixing rates of surface and deep oceanic waters, an important parameter in global carbon dioxide studies.

Nonlinear Optical Phenomena

The scope of ORNL's chemical physics studies based primarily on multiphoton optical techniques includes a variety of other topics. Extending the useful wavelengths of pulsed-laser sources is one area that is receiving much attention. We have done detailed studies of nonlinear "wave mixing" in gases, vapors, and gaseous mixtures. This interesting phenomenon occurs when intense laser beams traversing several types of media create new beams of light at frequencies that are mul-



Schematic diagram of the RIS-based "Maxwell Demon" used to sort and count ^{81}Kr atoms in groundwater samples.

tiples of the original laser frequencies or sums or differences of the frequencies of the incoming laser beams. Nonlinear wave mixing permits us to create new laser beams having wavelengths shorter than those available from currently existing laser dyes (i.e., ultraviolet wavelengths) and longer than those available otherwise (i.e., infrared wavelengths). Such extensions of existing laser technology are often required to provide a resonant wavelength needed in a particular application, such as the ^{81}Kr detection problem.

In doing these studies, we discovered new physical effects associated with nonlinear wave mixing in gaseous media. Indeed, because physicists are less accustomed to dealing with nonlinear phenomena, we often observe surprising behavior. For example, we have found that, under many circumstances, the new frequencies generated can interfere with processes caused by the initial laser beams. Under the conditions at which the nonlinear wave mixing occurs, the interference effect can be strong enough to completely prevent laser excitations in certain classes of atomic transitions. Another type of interference from new frequencies generated within an atomic medium limits the efficiency of generating ultraviolet beams, while yet another manifestation causes one of the generated beams to travel only backwards (i.e., in a direction opposite to that of the laser beam used to generate the process).

Once understood, these nonlinear optical phenomena can be useful. Based on nonlinear wave mixing studies, Garrett, Payne, and Wanda Ferrell devised and implemented a method for simultaneously determining atomic oscillator strengths, absorption coefficients, and linear indices of refraction for very-short-wavelength ultraviolet light generated in noble gas mixtures. In separate work, a three-photon resonant type of RIS scheme was used to measure pressure-induced broadening and shifting of atomic resonant transitions in extreme pressure regimes that are inaccessible by conventional techniques. Some of these nonlinear optical techniques are being used to develop methods for remotely detecting minute concentrations of airborne molecules.

Studies of Rare Processes

Sensitive detection systems based on multiphoton ionization can also be used to study processes that occur at very low probability (analogous to our detection of species that are present in low concentrations). In this context, we can produce low-probability excitations of very high-energy molecular states through a novel two-photon process that combines one low-intensity, high-energy photon (ninth harmonic of a Nd:YAG laser) plus one photon from a high-intensity tunable laser beam. Then, by making RIS measurements, we can gain information about spectral positions (energy levels) of the new molecular states produced and, for the first time, measure the absolute two-photon transition probabilities. This information could help solve the complementary problem of developing more sensitive detection methods for some chemical species (e.g., perhaps a more sensitive plastic-explosives detector for use in airports).

Some of the laser-based techniques developed at ORNL over the past 10 to 12 years have been used in fields as diverse as climatology, geology, and solid-state electronics. New experiments aimed at detecting solar neutrinos, observing quarks, and producing and detecting superheavy atoms are made feasible by the methods and modifications of RIS. We will continue to explore the new research opportunities provided by these promising new laser-based technologies. ornl

W. Ray Garrett is head of the Chemical Physics Section of ORNL's Health and Safety Research Division. He came to ORNL in 1966, three years after receiving his Ph.D. degree in physics from the University of Alabama.

Marvin G. Payne is leader of the Photophysics Group in the Chemical Physics Section. An ORNL staff member since 1972, he holds a Ph.D. degree in physics from the University of Kentucky. He and Sam Hurst are coauthors of the book *Principles and Applications of Resonance Ionization Spectroscopy.*

Surface and Submicron Physics

By Thomas L. Ferrell and Edward T. Arakawa

ORNL researchers invented a photon scanning tunneling microscope that images biological samples.

One of the ambitions of the scientific community over the past several decades has been to "visualize" individual atoms by imaging the electron cloud around the outer part of the atom at a resolution that is a small fraction of the atomic size. In the early 1980s, the development of the scanning tunneling microscope (STM)—a revolutionary type of electron microscope that offers a resolution as much as 100 times that of the best scanning electron microscopes and that received a 1986 Nobel Prize for Physics—allowed us to achieve this goal.

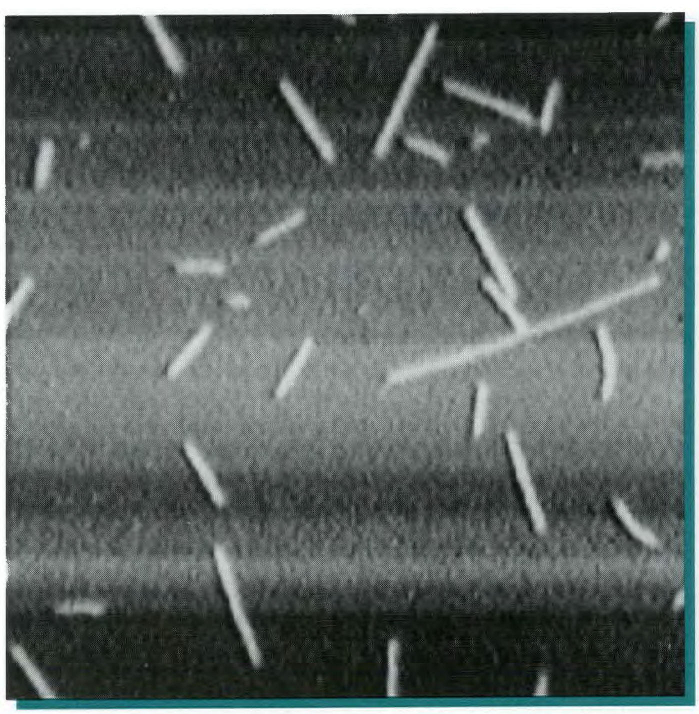
Electron STM

The first version of an STM at ORNL, and in the entire southeastern United States, was constructed in 1986 by the Liquid and Submicron Physics (LSP) Group of ORNL's Health and Safety Research Division (HASRD). This group's research interests range from basic physical phenomena to the development of practical instrumentation for biological, chemical, and environmental applications. Our first STM images of the electron distribution (electron density) around individual atoms were obtained in late 1986 for the surface atoms of pristine silicon in ultrahigh vacuum. Silicon was a prime target because of its importance in the microelectronics industry.

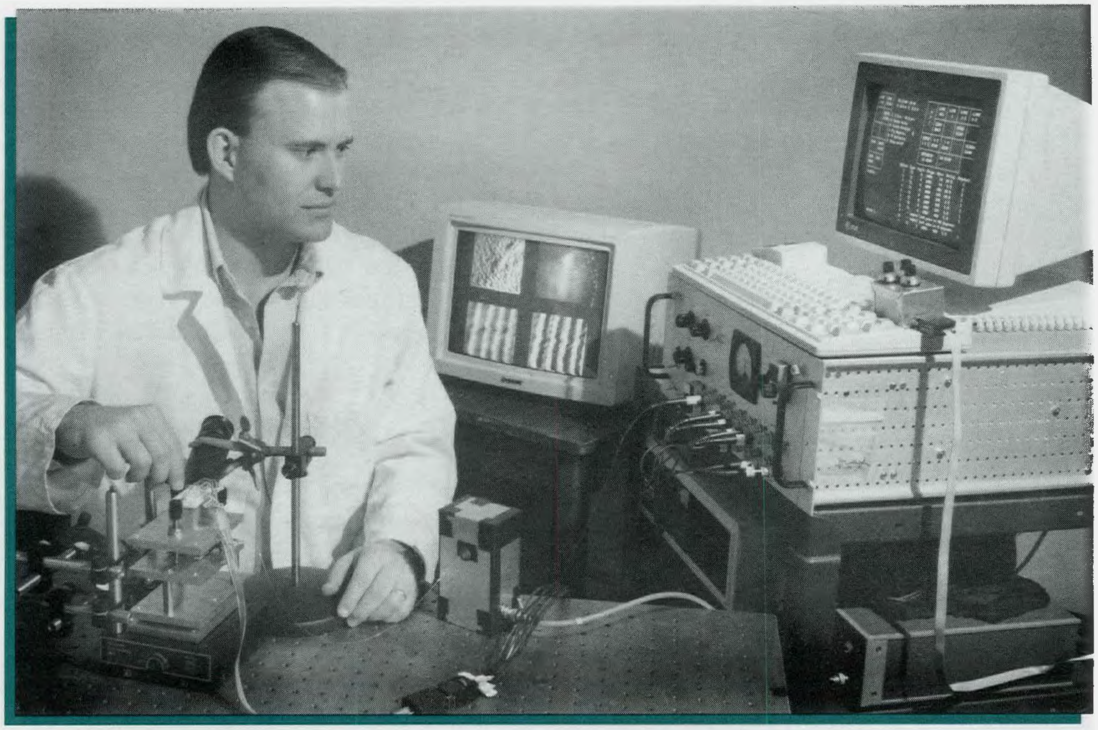
Today ORNL's STM comprises four instruments, three of which operate under air, water, liquid nitrogen, or other fluids. With these, we have examined atoms of metals, graphite, and conducting polymers; silane molecules on highly oriented pyrolytic graphite; and tobacco-mosaic viral (TMV) organisms on gold-coated mica (shown on this page). The STM achieves a somewhat poorer resolution on biological samples than on inorganic materials because biological matter tends to bind electrons very tightly. Fortunately, the resolution (0.1 nm) is high enough to clearly identify the TMV. As improved STM techniques are developed for biological materials, it may become possible to use STM to sequence the human genome at unprecedented rates and resolutions.

Improved SERS Technique

The LSP Group shares with HASRD's Physics of Solids and Macromolecules Group in research on the rapid identification of ultralow levels of chemical compounds on surfaces. Technologies available for this in the past have been unsatisfactory. Mass spectroscopy is very sensitive and discriminates well, but it is a relatively slow and expensive process. Infrared spectroscopy is relatively rapid but, in many cases, is not readily applicable for detecting the smallest quantities



Tobacco mosaic virus imaged in air by ORNL's electron scanning tunneling microscope.



Robin Reddick, a UT student, helped develop the photon scanning tunneling microscope at ORNL.

required. Neither method is very useful for aqueous samples.

In the past ten years, surface-enhanced Raman spectroscopy (SERS), which relies on detection of color shifts in visible light, has been the method used to rapidly identify single layers of organic molecules adsorbed on a silver-coated surface. Our research has developed microstructured silver substrates that greatly enhance the sensitivity and versatility of SERS instrumentation. We have obtained sensitivities more than a million times better than that of conventional methods, and we demonstrated that a number of toxic compounds can be detected in trace amounts by depositing a microstructured silver surface on an optical fiber. The fiber is also useful as an extended probe in portable instruments for field studies to screen water supplies or chemical dumps for toxic compounds. Other potential applications of our SERS improvements include drug screening, counterfeit deterrence (by tagging documents with SERS-detectable compounds), and monitoring of airborne pollutants.

Photon STM

Recently, Bruce Warmack and Tom Ferrell of HASRD and Robin Reddick, a University of Tennessee student, developed a photon scanning tunneling microscope (PSTM), which is the optical analog of the electron STM.

Although the PSTM has one-thousandth the resolution of the STM, it has an important advantage over the STM. Unlike the STM, the PSTM does not require the samples to be conducting or coated with conducting materials. As a result, it can be used to characterize optical and other electrically insulating surfaces, such as those found on biological samples. The first biological sample imaged by the new microscope was *E. coli* bacteria, rod-shaped organisms found in the human stomach that have been used to clone new genes (see photo on next page).

The PSTM has imaged details as small as one-sixth of a wavelength of visible light (~100 nm), several times better than the theoretical resolution limit for optical microscopes. By modifying the

PSTM to use ever smaller wavelengths, the developers hope to increase its resolution ten times. This improvement would make the new instrument competitive with a typical scanning electron microscope, which can visualize details as small as 10 nm. However, unlike the scanning electron microscope, the new optical system will not require a vacuum for the samples. Thus, samples can be examined in the air—an important advantage if they are alive.

In the STM, electrons flow freely between atoms at a sample's surface and the tungsten atoms at the probe's needle tip, a phenomenon called tunneling. The electron flow, which varies according to the distance between a surface atom and the needle tip, causes fluctuations in current along the needle. To keep the current constant, a piezoelectric crystal contracts or expands, changing the height of the tip with respect to the sample. These current changes are measured by an ammeter and the changing tip heights are correlated with surface elevations by a computer, to reveal the relative locations of the surface structures, often at atomic resolution.

In the PSTM, the source of photons is a helium-neon or helium-cadmium laser. The light is internally reflected in a prism at the prism base. The sample is placed outside the prism on the base. Nearby is an optical fiber probe with a sharp tip. The photons from the incident laser beam "tunnel" between the sample and tip, which transmits them to a detector. The intensity of the tunneling photons is varied by the presence of the sample because of varying elevations in the sample surface.

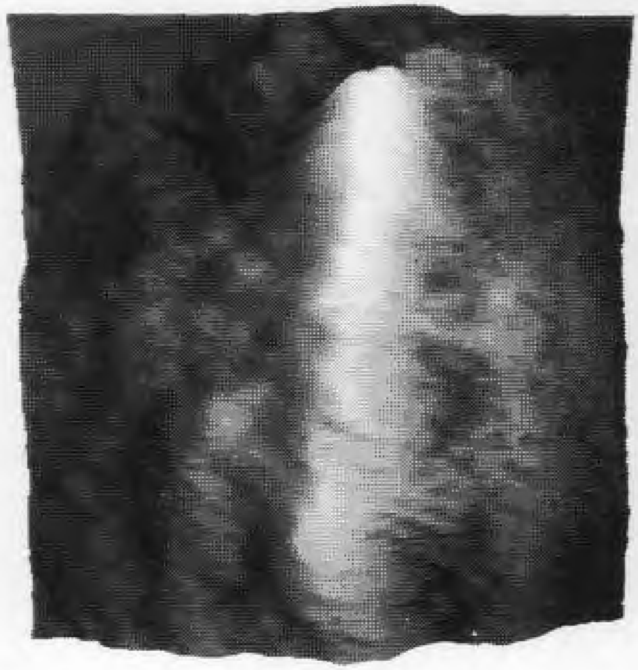
The changes in photon intensity are translated into electrical signals by the detector (a photomultipler tube), which is connected to an electronic circuit that adjusts the separation between the optical fiber tip and sample to maintain a constant current. The tip height at each scan point is recorded and stored in memory by a computer, which uses the information to construct a three-dimensional image of the surface structure. In contrast, most microscopes yield only two-dimensional images.

The PSTM can also provide spectroscopic information that permits chemical mapping of the surface composition. In addition, the tip may be used to make high-resolution masks for production of integrated electronic circuits such as computer chips.

A patent application has been filed on our system. This and our other developments are steps toward our ultimate goal of probing biological and electronic materials molecule by molecule. ornl

Thomas L. Ferrell is a research staff member of HASRD's Liquid and Submicron Physics Group and a recipient of the 1986 Martin Marietta Inventor's Award.

Edward T. Arakawa is leader of HASRD's Physics of Solids and Macromolecules Group, a fellow of the American Physical Society and the Optical Society of America, and a recipient of a Martin Marietta Energy Systems Inventor's Award in 1987 and an I•R 100 award in 1986.



This rod-shaped *E. coli* bacterium is the first image of a biological sample taken with the new photon scanning tunneling microscope.

Radiation-Induced Interactions in Biological Materials

By Harvel A. Wright, James E. Turner, and Robert N. Hamm

When a charged particle, such as an electron or alpha particle, travels through biological material, it physically interacts with the atoms and molecules of the material. These physical interactions result in the transfer of energy from the particle to the material and, in general, cause excitations or ionizations of the surrounding atoms and/or molecules. Secondary electrons produced by such ionization events may, themselves, have sufficient energy to cause additional ionizations and excitations.

These physical events occur very rapidly. In local regions of a track, the physical interactions produced by a primary charged particle and all of its secondaries are complete within about 10^{-15} s (one millionth of one billionth of a second). The initial physical changes are followed by ion-molecule reactions that result in the production of chemically reactive ions and radicals that are present ~10^{-12} s (one millionth of one millionth of a second) later. Diffusion and chemical reactions within the track or path of a charged particle begin at this time and are generally complete in about 10^{-6} s.

These rapid reactions in irradiated tissue produce changes that can contribute to cancer development decades later. A detailed understanding of these physical and chemical interactions with biological molecules will help us understand the mechanisms involved in the production of cancer and other radiation-induced biological damage. For this reason, physicists, chemists, and biologists have been working for decades to understand the details of this complex sequence of events.

Little is known about these interactions in condensed matter (liquid or solid phases) because many of the physical and chemical events are not amenable to direct measurement. In the gas phase, a charged particle can interact with a single atom or molecule, subsequently scattering at a lower energy and in a new direction. A detector can then record the energy and direction of the incident particle as well as the energy, direction, and identity of the other products of the interaction. However, in the condensed phase, the interaction of an atom or molecule with a charged particle is influenced by the presence of its neighbors. In addition, a charged particle may interact almost simultaneously with many atoms or molecules of material in the condensed phase.

Monte Carlo Simulations

In the mid-1970s we, together with Rufus Ritchie, also in the Biological and Radiation Physics Section of ORNL's Health and Safety Research Division (HASRD), decided to try something that had never been done before—to develop cross sections (probabilities) for the interactions of charged particles with liquid water. (Cross sections had been developed for gaseous water, but never for the liquid phase.) We then used Monte Carlo techniques (named for the city famous for its games of chance) to produce a computerized simulation of the events that would occur as a charged particle passes through liquid water. Water is, of course, a medium of great biological relevance, because cells are composed mostly of water.

To develop the needed cross sections, we first had to determine the specific energy losses that might occur. Pioneering measurements of liquid water's absorption and reflectance of photons in the vacuum-ultraviolet region of the spectrum had recently been made by Bob Birkhoff of our section, now retired, and Linda Painter, now professor of physics at the University of Tennessee. Starting with these optical measurements, we developed a model for the specific energy-loss events that would occur and estimated the cross sections for each type of event. Using these cross sections, we developed a Monte Carlo computer code that randomly selects the distance a particle travels to a collision with a water molecule, the particle's energy loss as a result of the collision,

ORNL physicists devised a new particle-detection system to help identify mechanisms of radiation damage to biological molecules.

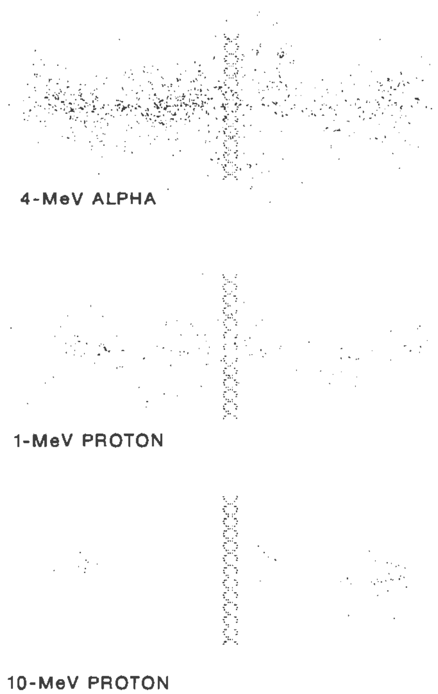
and the type of event the energy loss produced. The code thus simulates the transport of a primary charged particle and all of its secondaries through liquid water and records the position and identity of every physical event that occurs.

Working in collaboration with radiation chemists John Magee and Aloke Chatterjee of the Lawrence Berkeley Laboratory, we also developed a model for determining the identity and position of each reactive chemical species (ion or radical) produced by each of the physical interactions. Another model was developed to describe the diffusion of these reactive chemical species through the water, as well as their interactions with each other and with other molecules in solution.

Alphas, Protons, and DNA

In biological materials, a charged particle traveling through the nucleus of a cell may interact directly with a molecule, such as DNA, or it may interact with the surrounding medium to produce chemical ions or free radicals that can diffuse and interact chemically with the DNA to produce indirect chemical damage. Biological systems have an amazing intrinsic ability to repair the damage to a DNA molecule. However, if both DNA strands are involved, or if the damage is extensive, the system may not be able to repair itself, and the damage is permanent.

In general, high-LET (linear energy transfer) radiation produces a denser track of these harmful ions and radicals and causes more biological damage than low-LET radiation. The figure on this page shows the code-calculated tracks of three charged particles, superimposed on idealized segments of a DNA molecule (shown as two helical strands)The black dots in the track represent the positions of reactive chemical species produced by the particle. It is easy to see that much more extensive damage may result from the 4-MeV alpha particle (high LET) than from the 10-MeV proton (low LET). In fact, the latter might pass directly through the center of the DNA without producing a single collision event.

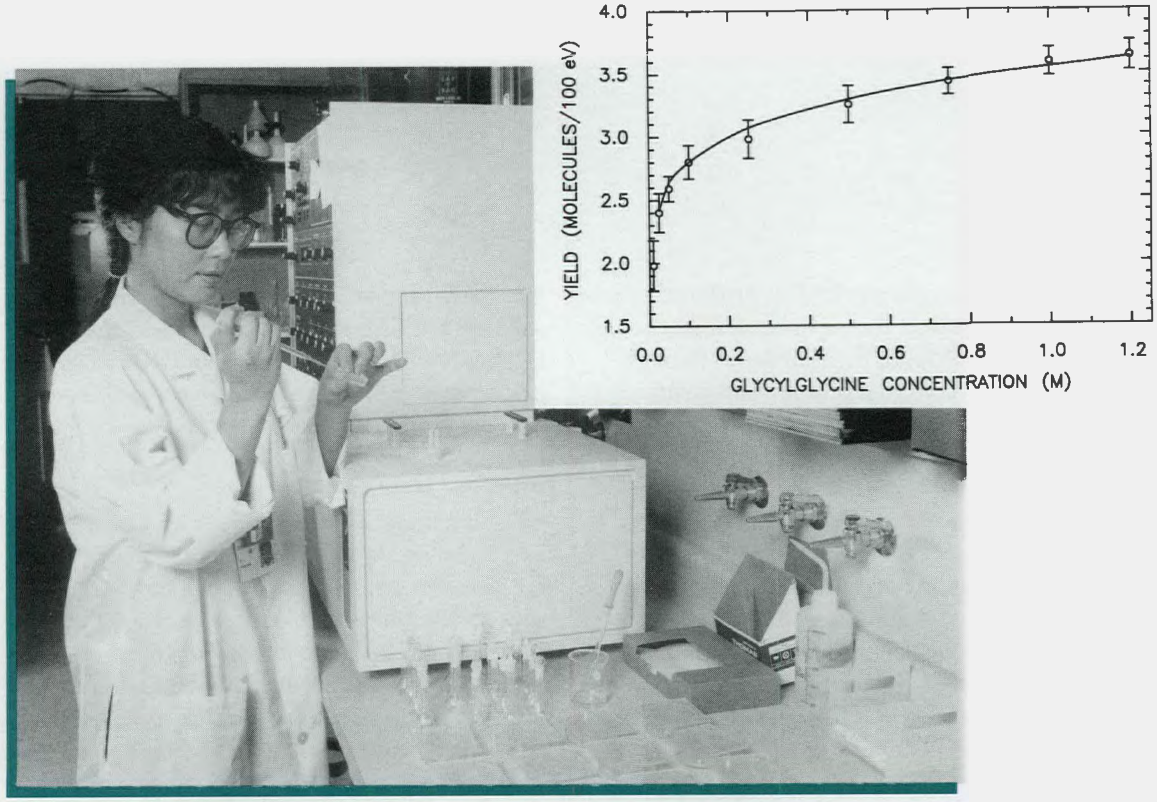


These code-calculated tracks show reactive chemical species (black dots) produced as charged particles pass through idealized segments of a DNA molecule (double helix). The faster-moving, high-energy (10-MeV) particle obviously causes fewer collision events affecting the DNA.

Measuring Damage to Molecules

We have recently taken another major step toward identifying the mechanisms of radiation damage to biological molecules. A new experimental program is aimed at measuring the damage to specific molecules irradiated in aqueous solution and comparing these data with calculated damage for the same conditions. For the initial work, we chose the dipeptide glycylglycine. A hydrated electron (one of the reactive chemical species produced in charged-particle tracks) can interact with glycylglycine in water to produce ammonia. The yield of ammonia depends on both the concentration of the glycylglycine and the LET (or density of reactive species in the track).

Hiroko Yoshida, of our section, and Bruce Jacobson, of the Biology Division, have measured ammonia production in solutions of glycylglycine at various concentrations during irradiation by 250-kVp X rays. While working with us on his Ph.D. dissertation, Wesley Bolch, a DOE Fellow from the University of Florida (now on the Texas A&M University faculty), calculated (1) the energies of electrons that would be produced by these X rays in water and (2) the ammonia production



Hiroko Yoshida (shown here) and Bruce Jacobson, of the Biology Division, are measuring the ammonia produced in solutions of glycylglycine exposed to X rays. Their data (circles plotted in inset graph) show excellent agreement with the calculated results (smooth line in graph).

that would result from the transport of these electrons. There is excellent agreement between Bolch's calculations and Yoshida and Jacobson's experimental results (see figure above).

Our next step is to study more complex molecules, such as oligonucleotides and segments of double-stranded DNA. This approach offers great promise for identifying the specific mechanisms of radiation-produced molecular damage in biological systems. Our program represents the first successful linking of the physical, chemical, and biological interactions resulting from irradiation of an aqueous system.

Current Research

Our section has recently initiated a program to apply these techniques to the study of radon, currently an indoor-pollution problem of considerable concern. The decay chain of radon includes daughters that emit alpha particles. We plan to study the specific mechanisms by which radon-produced alpha particles cause damage to biological molecules and compare the damage quantitatively with that produced by other types of radiation. In the long run, this approach may provide our best hope of getting quantitative estimates of the risk of exposure to low levels of radon.

A recent development in measuring the structure of charged-particle tracks complements this work. About three decades ago, the micrometer-sized nucleus of a biological cell was identified as the cell site most sensitive to radiation damage. The field of microdosimetry emerged to measure energy deposition in sites of this small size. Low-pressure ionization chambers were developed to simulate the quantity of matter in micrometer-size volumes of unit-density materials (such as biological tissue). Since that time, it has become increasingly apparent that the dimensions of the most sensitive target area are in the nanometer range—the diameter of the DNA molecule. Attempts to measure energy deposited in such minute volumes have heretofore been unsuccessful.

With assistance from Sam Hurst (now retired from ORNL) and Scott Hunter (currently working at GTE), we have recently designed a device that can approach nanometer resolution in measuring the structure of charged-particle tracks in a gas. When a charged particle traverses the gas in a chamber, it produces ionizations, which produce secondary electrons that may themselves produce more electrons. Thus, the track will contain many electrons distributed along the track where energy transfers occur. The positions of the electrons in the track can then be detected optically by applying a high-frequency alternating electric field. This causes the electrons to oscillate about their positions, colliding with molecules of the chamber gas. These collisions excite the gas molecules,

Operating on a "shoestring budget," researchers Bob Hamm, Harvel Wright, and Jim Turner (left to right) and their colleagues have demonstrated the feasibility of their particle-detection system with this improvised equipment setup. (Inset shows an optical image of an alpha-particle track produced by their system.)

which fluoresce during decay back to their ground states, pinpointing the positions of the electrons. Two optical detectors at right angles to each other are used to locate the electrons, each detector producing a two-dimensional projected image of the track.

We have begun construction of our detector system and have already obtained photographs of alpha-particle tracks. Using a car battery as a power source and the crude experimental setup shown here, we were able to obtain encouraging preliminary results (the inset shows one of our photographs of an alpha-particle track). We are currently seeking funding support for this research that would allow us to obtain sensitive two-dimensional optical sensors to resolve the structure of tracks. If our system proves successful in imaging track structure with a resolution approaching nanometer dimensions in unit-density material, this may usher in a new field of "nanodosimetry." The device will also have applications in neutron dosimetry, laser-beam characterization, X-ray imaging, and many other aspects of energy loss accompanying charged-particle penetration. ornl

Harvel A.Wright received his Ph. D. in mathematics from the University of Tennessee, after joining the ORNL research staff in 1962. Since 1974, he has been head of the Biological and Radiation Physics Section of the Health and Safety Research Division. He has worked as a visiting scientist at the European Center for Nuclear Research (CERN) and other foreign institutions and is the author of more than 100 publications in the field of radiation physics.

James E. Turner was a Fulbright Scholar in Germany and received his Ph.D. in physics from Vanderbilt University. Before joining ORNL in 1962, Turner taught physics at Yale University and served with the AEC in Washington, D.C. Appointed a Martin Marietta Energy Systems Corporate Fellow in 1988, Turner is the author of three textbooks and more than 200 open literature publications. He is also a fellow of the American Physical Society, the American Association for the Advancement of Science, and the Health Physics Society and served on the editorial boards of two professional journals.

Robert N. Hamm received his Ph.D. in physics from the University of Tennessee in 1967. He has been a research staff member of ORNL's Health and Safety Research Division (formerly Health Physics Division) since 1963. He is the author of ~100 publications on radiation physics and serves on the editorial board of *Radiation Research.*

Ranking Chemical Toxicity

By Annetta Watson, Troyce Jones, Clay Easterly, and Bruce Owen

How toxic is "toxic?" What is a safe drinking water concentration for any one of the hundreds of new compounds being developed annually for commercial use as insecticides, plasticizers, industrial cleaners, and solvents? How does a federal, state, or local regulator on a limited budget decide which hazardous waste site should have top priority for cleanup? These questions are not new; some very sophisticated thinking on this subject took place during the 16th century when the Swiss physician and alchemist Paracelsus (1493–1541) stated, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy."

Our Health Effects and Epidemiology (HE&E) Group in the Health and Safety Research Division helps define that "right dose" for the Environmental Protection Agency (EPA), the Department of the Army, and the U.S. Air Force.

A critical convergence of national and international events pushed ORNL researchers into the arena of chemical toxicity. Historically, the Laboratory's efforts had been in line with the main DOE (ERDA, AEC) objective of developing the peaceful uses of atomic energy. Thus, considerable expertise was developed at ORNL in assessing health issues related to nuclear power. However, because of the Arab oil embargo and energy crisis of the 1970s and the de-emphasis of nuclear energy during the Carter Administration, the backbone of the U.S. energy supply was seen as coal and its liquid and gaseous by-products. This emphasis required a shift in attention from the relatively well-studied radiological effects of nuclear power production to the sparsely studied health effects of chemical pollutants produced by the use of fossil fuels.

Technological efforts to design and operate facilities for producing liquid and gaseous fuels from coal had to be accompanied by studies to ensure that workers and public health were protected. These facilities generated poorly characterized intermediate and waste products ("black goop"), which were suspected of being quite toxic and possibly carcinogenic. As chemical analyses of these wastes began, it became clear that the cumbersome regulatory process was unprepared for the onslaught of chemicals requiring regulation. The HE&E Group's task was to provide exposure guidelines concurrent with planned construction and operation of these facilities.

Different but complementary ideas were being nurtured independently by Troyce Jones and Phil Walsh (former leader of the HE&E Group); the approach that ultimately developed was radically different from historical methods for assessing toxicological hazards. There simply wasn't time for expert committee debate and numerical evaluation of complex, chemical-specific, mathematical models. We had to rely on intensive use of data in an attempt to provide a methodology that could be generally applied.

This generalized approach, which minimizes the role of complex mathematical models, also reduced the uncertainty factor in assessments, because a major portion of the uncertainty in risk analysis comes from the mathematical analogy. The key to our approach was an old concept from pharmacology and radiation protection—relative potency.

Relative Potency

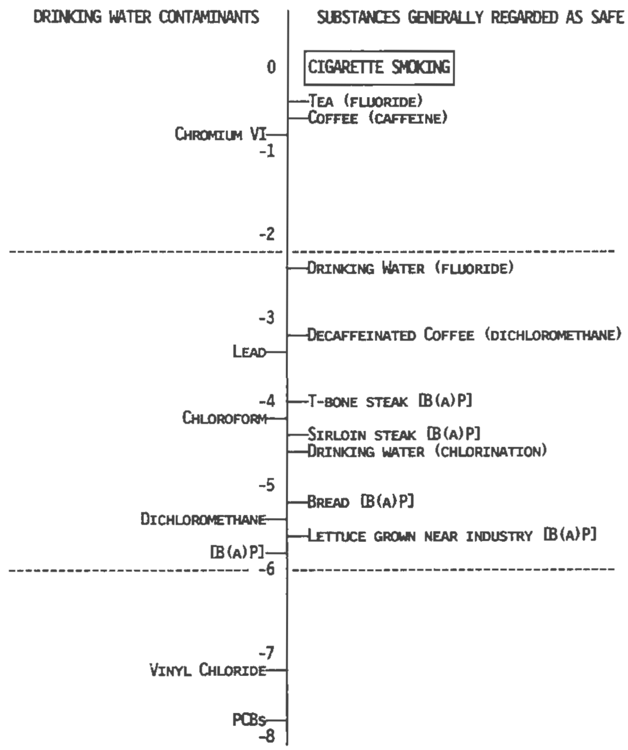
In a relative potency approach, the toxic effects of the substance being evaluated are compared to those of a well-studied chemical, considering all levels of biological data. The potency of the newly identified components of the black goop can thus be compared with the potency of a well-studied "reference" chemical and with a variety of the chemicals appearing for one reason or another in the daily life of nearly everyone, many of which are generally regarded as safe at environmental levels. For example, relationships in the logarithmic scale of relative drinking water contaminant hazards shown on the next page suggest that vinyl chloride and PCBs may be overregulated. Chromium VI may be underregulated, relative to other EPA criteria, but not relative to consumption of tea or coffee, as shown on the chart here. The PCBs, with a relative rating of minus 8 on the scale, pose the lowest actual health hazard of all the substances rated.

By incorporating information about common environmental exposures that are generally regarded as safe, equally safe levels of newly identified chemicals can be estimated. This relative method is in contrast to "absolute" methods of assessment, which are characterized by committee deliberations where great emphasis is placed on choosing the single sentinel experiment that can be used to evaluate a mathematical model. The model most often used at EPA is the "linearized multi-stage model," which is compared with test dose-effects curves in the near-toxic range from the selected experiment and then extrapolated to very low doses that may be a millionfold lower. In so doing, untested assumptions can introduce significant uncertainties. The magnitude of the uncertainty can greatly exceed the magnitude of the hazard at environmental concentrations.

Although a high level of precision is suggested by the absolute method's complex mathematical relationships and careful data selection, the underlying assumptions and methods provide only the seductive illusion of accuracy. The lack of standardized reference points and the inability to know the range of uncertainty of the hazard from a particular exposure make accurate assessment impossible.

In developing an alternative to the absolute type of assessment, we are facing some different questions because the method is new. What problems can be addressed with this approach? How well will this concept be accepted? How good a predictor is the method? These questions are being asked by those attempting to use the relative potency concept in real-world situations. The advantage of this approach is that it elucidates simple, intuitive relationships between chemical agents and does not obscure the comparisons of measured effects with unnecessary use of complex mathematical models.

The illustration on the next page depicts the two approaches to estimating the hazard associated

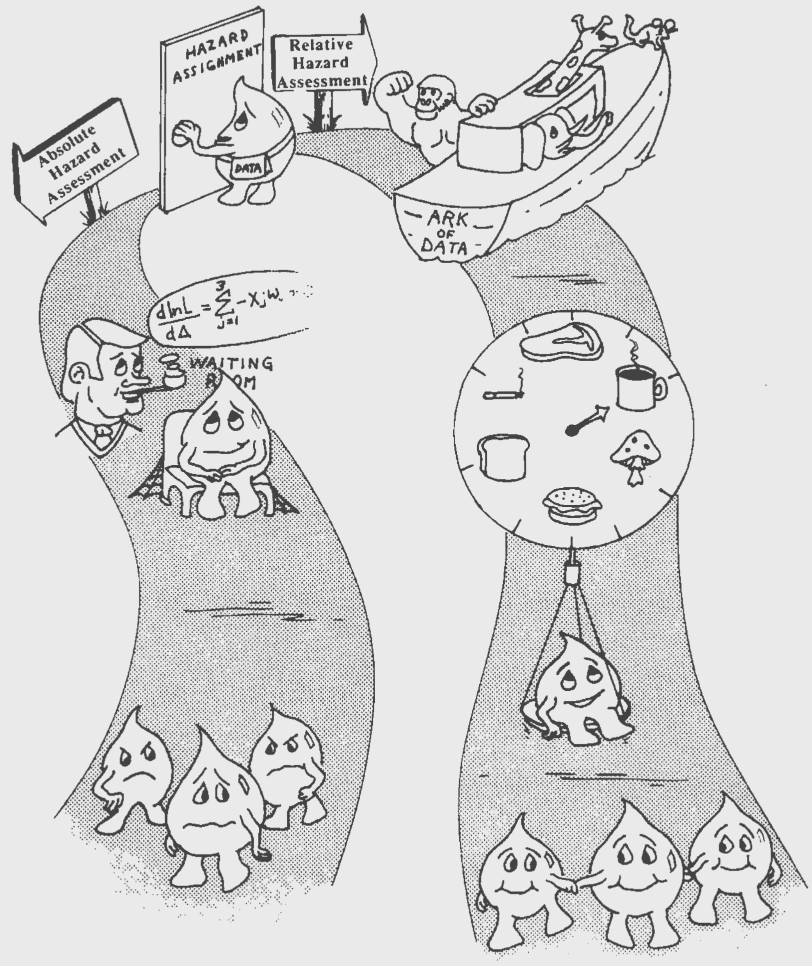


Some surprises are seen in this logarithmic scale showing relative lifetime hazards from smoking one pack of cigarettes daily (most hazardous) and from average human ingestion of common drinking water contaminants and substances generally considered safe.

with a new or untested chemical. The absolute assessment path (left) relies on complex extrapolation models applied to a small subset of the available test data. Time-consuming deliberation by expert committees may still assign the test chemical to the wrong "neighborhood" of hazardous substances. The relative hazard assessment path (right) evaluates all available (usually laboratory animal and cell culture) test data and minimizes the use of extrapolation models based on untestable assumptions. When its toxicity is then compared to the hazards associated with commonly encountered substances, the test chemical can be assigned to a "neighborhood" of reasonably equivalent hazards.

Defense Priority Model

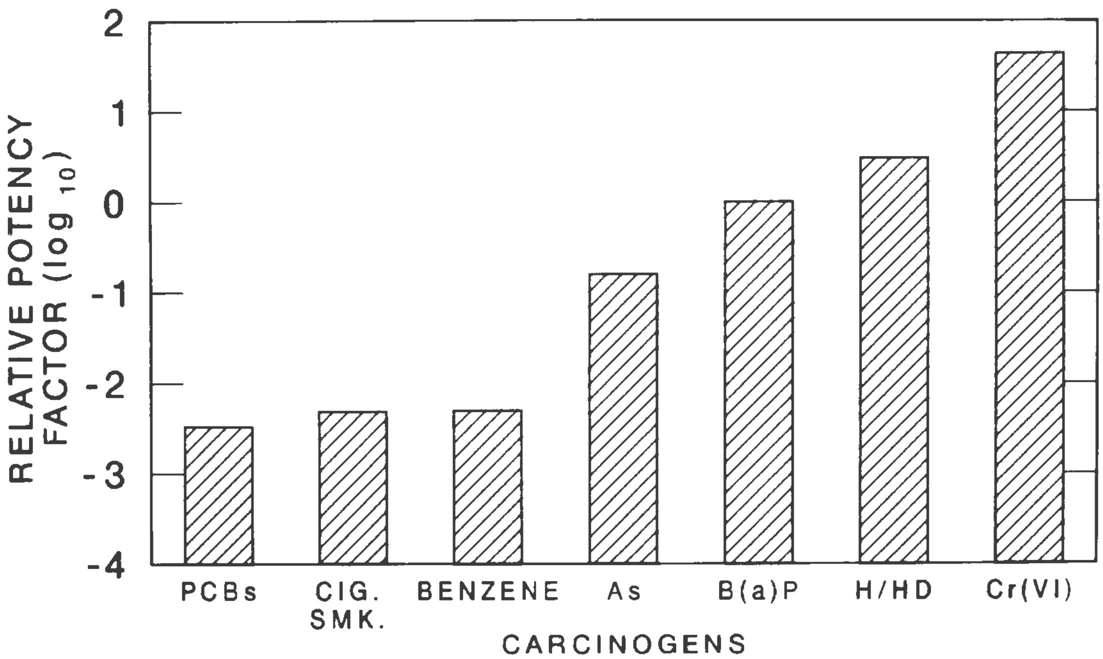
The U.S. Air Force Installation Restoration Program requires that inactive hazardous material disposal sites on Department of Defense (DOD) property be identified, evaluated, and ranked according to their potential adverse effects on human health and the environment. Because of the large number of sites, it is necessary to prioritize them for investigative studies and actual cleanup. Extensive collaboration between the Environmental Sciences Division and HASRD resulted in the development of the "Defense Priority Model," which makes use of site-specific monitoring data, toxicological benchmarks, and bioaccumulation factors to score each site on a systematic basis. The human health hazard analysis built on the earlier experience of the Fossil Energy Technology Environmental Program, which had developed some rudimentary concepts.



More than 200 hazardous chemicals and mixtures in their inactive disposal sites were identified by the Air Force as needing evaluation. Because regulatory or guidance criteria were available for only about 50 of the compounds, we recognized the need to "validate" the relative potency approach in some way to give the sponsor confidence in our evaluations of the additional 150 compounds. We developed a defensible set of data-comparison rules, called the Rapid Screening of Hazard (RASH) method and used it to evaluate more than 250 materials. During this process, we identified certain data gaps and refined the comparison rules into a flexible chemical scoring system.

Initially, we scored the potencies of the various chemicals and mixtures by comparing them to the potency of benzo(*a*)pyrene [B(*a*)P], a polynuclear aromatic hydrocarbon found in coke oven emissions. Then, where possible, we compared our rankings with the determinations derived by committees of experts, such as EPA's Carcinogen Assessment Group and the American Conference of Governmental Industrial Hygienists. Our predictions using the RASH method were found to agree with those developed by expert committees (using the absolute method) and were consistent with the degree of variability observed for intercommittee comparisons. Based on our results, the fundamental concepts of relative potency have

Mustard tumorigenicity ~ B(a)P tumorigenicity



The tumorigenicity of sulfur mustard (H/HD) is nearly the same as that of B(*a*)P, as shown in this Rapid Screening of Hazard (RASH) analysis.

been well received by DOD, as demonstrated by their decision to incorporate the RASH method (as developed by Jones et al.) in their DOD site restoration effort.

Predictability of Short-Term Tests

A relative-potency framework can also be useful in research. It was recently helpful in addressing a very important question in toxicology: Can short-term tests be predictive of long-term carcinogen bioassays? Larry R. Glass, a doctoral student from the University of Texas Health Science Center at Houston, joined our group's effort to investigate this.

One of his main concerns was the problem of chemical mixtures. No current data base or theory allows toxicity predictions for a mixture of chemicals. Thus, regulation of mixtures must be based on either chemical analysis, which assumes that chemicals do not interact, or biotesting, which requires the use of long-term bioassays. However, the prohibitively high costs of long-term bioassays for the many chemical mixtures present at waste and effluent sites encourages the use of less-expensive short-term bioassays. Despite attempts by many eminent biologists and toxicologists, no one has suggested a satisfactory method for interpreting data from short-term bioassays in the context of long-term health effects for mixtures.

However, Glass has determined that a modification of RASH might serve this purpose, deriving a data set that may be used to systematically investigate the relationships between long- and short-term bioassays. His results suggest that a battery of short-term tests may provide useful information for health-hazard ranking. There are unanswered questions, such as how to identify the "best" battery of bioassays, but these are encouraging results.

Chemical Stockpile Disposal Program

Destruction of the nation's stockpiles of obsolete chemical weapons by incineration is scheduled for completion in 1994. These weapons contain the blister agent sulfur mustard—a recognized carcinogen that was first used as an antipersonnel agent near Ypres on the Belgian front in 1917. Because of its status as a warfare agent, sulfur mustard has not been widely evaluated in standard laboratory testing systems. Nevertheless, the Department of the Army needed an estimate of the additional cancer risk posed to the surrounding population from operation of the proposed mustard agent incinerator.

Using a modified form of the RASH approach, Watson compared laboratory test data for sulfur mustard with toxicity data for numerous reference compounds and elements. She found that the ability of sulfur mustard (identified as H/HD in the figure on p. 189) to produce cancerous tumors nearly matched the tumorigenic potential of B(*a*)P. Other well-known carcinogens were ranked as less potent (e.g., benzene and cigarette smoke condensate) or more potent (e.g., chromium VI) than B(*a*)P.

This finding allowed Watson to calculate an estimate of excess lifetime cancer risk posed by hypothetical incinerator releases of sulfur mustard. These results were used by the Centers for Disease Control (U.S. Department of Health and Human Services) in developing recommendations on long-term exposure to low doses of chemical warfare agents that were published in the *Federal Register* of December 1987.

ORNL Applications

A recent application of the RASH approach has been the preparation of a toxic hazard ranking for the proposed upgrade of aboveground tank storage of hazardous materials at ORNL (the ORNL Tank Compliance Program). The tanks in question contain a variety of chemicals, some of which are well-characterized (e.g., sulfuric acid) and some of whose toxicities are unknown (e.g., dyes). In collaboration with staff of the Operations Division and the Environmental and Health Protection Division, we have prepared relative potency scores for 33 individual compounds and 22 mixtures. By integrating these data, we identified the tanks that pose the greatest risk to the environment and ranked them according to need for remedial actions.

Developing and applying novel methods for health hazard assessment has been a major emphasis of our HE&E Group. Staff members are working to improve the quality of assessment methods by (1) systematically adapting the science of toxicology and hazard assessment to address practical needs of establishing regulatory standards and control actions, (2) questioning widely accepted assumptions, and (3) reducing dependence on mathematical models that cannot be tested. Because "necessity is the mother of invention," it is not surprising that the need to solve practical problems related to health assessment has motivated us to be innovative. Our relative potency approach has been successful in solving practical problems at ORNL and other agencies, helping to set priorities for allocating scarce resources, identifying relatively underregulated or overregulated chemicals, and providing tools to enhance experimental design in basic research projects. ornl

Annetta Watson is a research staff member in the Health Effects and Epidemiology Group of the Health and Safety Research Division. She became an employee in 1977 after completion of her thesis research as an ORAU Radiation Science and Protection Fellow; she has a Ph.D. degree from the University of Kentucky.

Troyce Jones, a research staff member of HASRD's Health Effects and Epidemiology Group, came to ORNL in 1963 from the Missouri School of Mines and Metallurgy. He served on the Pennsylvania Governor's Blue Ribbon Advisory Panel for Health Research Studies and takes part in NATO sessions in support of the U.S. Defense Nuclear Agency.

Clay Easterly, leader of the Health Effects and Epidemiology Group, has worked at ORNL since 1973. He was awarded an AEC Special Fellowship in Health Physics to perform undergraduate work at the University of Tennessee, where he earned his Ph.D. degree.

Bruce Owen is a senior laboratory technician in the Health Effects and Epidemiology Group and has worked at ORNL since 1980. He has a B.A. degree in microbiology from the University of Tennessee, is a state-licensed clinical microbiologist, and will soon receive a master's degree in public health from the University of Tennessee.

Interspecies Extrapolation for Risk Assessment

Body-weight scaling may aid interspecies risk extrapolation.

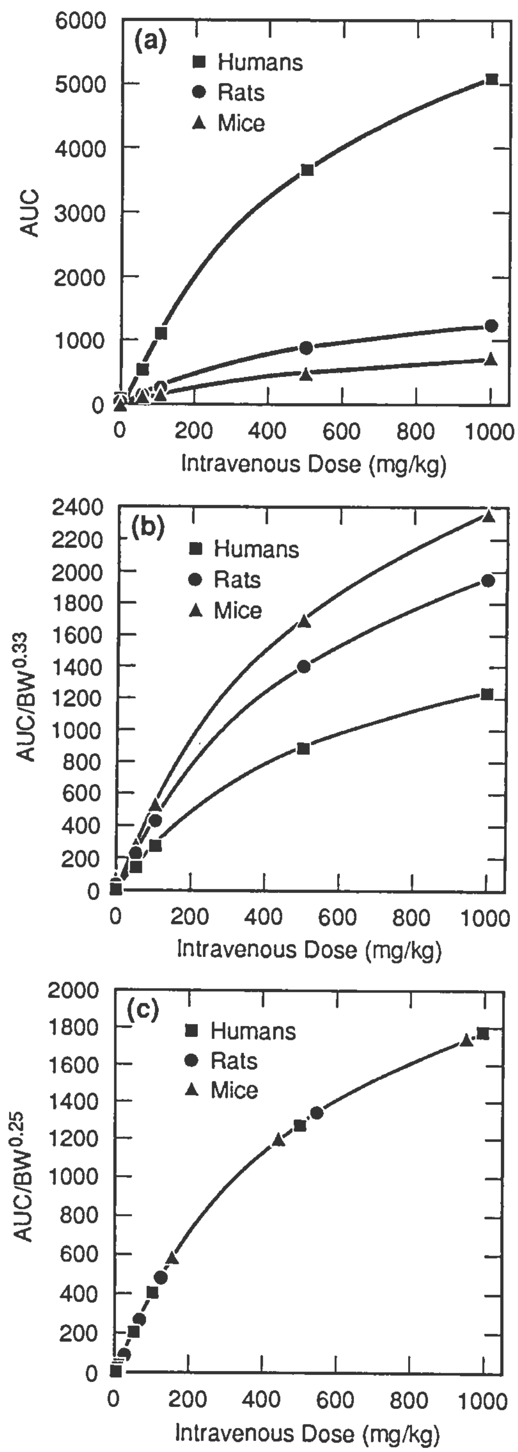
By Curtis C. Travis

In assessing human cancer risk from chemical exposures, a fundamental problem is how to extrapolate experimental results from animal species to humans. Detailed information on interspecific differences is lacking in many cases, so it is frequently assumed that results can be extrapolated on the basis of administered chemical dosage or exposure, standardized as either milligrams per kilogram of body weight per day (body-weight scaling) or milligrams per square meter per day (surface-area scaling). Neither of these extrapolation procedures is exactly correct, and when available, species-specific data should be used in risk assessment.

As we learn more about body systems and construct reasonably realistic pharmacokinetic models for describing bodily absorption, distribution, metabolism, and excretion of chemicals, we have more scientifically defensible justification for interspecies scaling. Biologically based models have been developed to provide a fairly accurate description of the pharmacokinetics of some parent compounds and metabolites in mice, rats, and humans (see p. 193). The controlling parameters in any given species are physiological (e.g., breathing rates, blood flow rates, blood volumes, tissue volumes), biochemical (partition coefficients), and metabolic. Because these parameters can be extrapolated across species, we believe the experimental risk data for chemical exposures can also be extrapolated.

It is generally agreed that the proper measure of dose to target tissue is the average tissue concentration of the toxic moiety, as measured by a mathematical entity known as area under the curve (AUC). Thus, the question of interspecies extrapolation of pharmacokinetics reduces to, "Is it possible to choose a measure of administered dose such that the AUC of the toxic moiety is the same in all species?" The following discussion illustrates how we derive an interspecies extrapolation rule for reactive metabolites that answers this question.

A reactive metabolite is a short-lived by-product of the metabolic process that interacts with the cellular constituents. The three figures here show



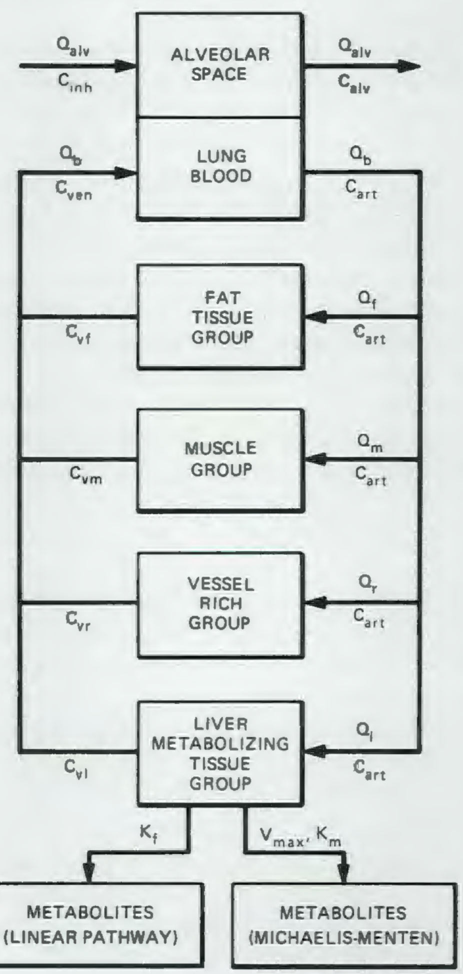
curves generated using a pharmacokinetic model to represent the concentrations of a reactive metabolite in livers of mice, rats, and humans, following an intravenous administered dose of a parent compound. In figure (a) on the preceding page, the AUC for humans is larger than that for rats, which is larger than that for mice. Thus, traditional body-weight scaling (mg/kg) would predict a *lower* than actual toxic effect of a reactive metabolite in humans, based on the animal data. In (b), the $AUC/BW^{0.33}$ for humans is less than that for rats, which is less than that for mice. Thus, traditional surface-area scaling ($mg/kg^{0.67}$) would predict a *higher* than actual toxic effect of a reactive metabolite in humans, based on the animal data. In (c), the $AUC/BW^{0.25}$ for humans, rats, and mice are identical. Thus, $AUC/BW^{0.25}$ is constant across species when the administered dose is normalized by body-weight scaling (i.e., mg/kg). To state this another way, the average tissue concentration (i.e., AUC) of a reactive metabolite will be constant across species if the administered dose is measured in $mg/kg^{0.75}$ Thus, we conclude that the 0.75 power of body weight (i.e., a modified surface-area scaling procedure) is a scientifically defensible interspecies scaling factor. ornl

Curtis C. Travis, who holds a Ph.D. degree in applied mathematics from the University of California at Davis, is coordinator of the Office of Risk Analysis in ORNL's Health and Safety Research Division, editor-in-chief of *Risk Analysis: An International Journal*, and author or coauthor of seven books. He is a recipient of the Distinguished Service Award from the Society for Risk Analysis.

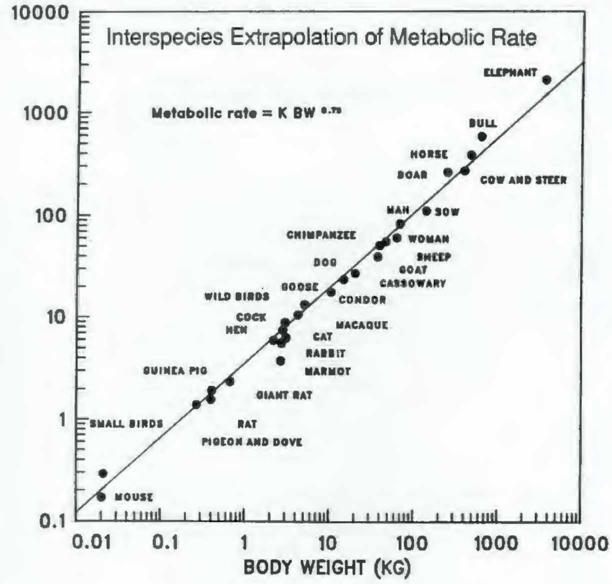
Physiologically Based Pharmacokinetic Models

The physiologically based models used for extrapolating risk data between species are derived by dividing the body into "compartments" connected by the arterial and venous blood flow pathways. The tissue groups generally include: (1) organs such as brain, kidney, and viscera, (2) muscle, (3) fat, and (4) metabolic organs—principally the liver. The models use actual measured data such as breathing rates, blood flow rates, blood volumes, and tissue volumes to describe the pharmacokinetic (or chemical breakdown) process. Coupling these data with chemical-specific information such as blood/gas partition coefficients, tissue/blood partition coefficients, and metabolic constants allows us to predict the dynamics of a compound's movement through an animal system. An advantage of this model is that by using the appropriate physiological, biochemical, and metabolic data (when known), the same model can be used to describe the dynamics of chemical transport and metabolism in any species, including mice, rats, and humans.

Because we cannot conduct toxic chemical experiments on humans, these models are our best means of extrapolating the experimental results of animal studies. Once a model has been calibrated using rodent data, it is theoretically possible to extrapolate the results to humans.



Allometric Scaling and Physiological Time



Allometric scaling is based on the suggestion that body size serves as the regulating mechanism for an internal biological clock, making the rate of all biological events constant across species, when compared on a unit physiological time basis. This suggestion is supported by numerous empirical observations that breath duration, heartbeat duration, longevity, pulse time, breathing rates, and blood flow rates are approximately constant across species when expressed in physiological time units. Physiological time (t') is defined in terms of chronological time (t) and body weight (BW) as

$$t' = t / BW^{0.25}$$

Thus physiological time is different for each species, whereas chronological time is the same. The merit of this concept is that all species have approximately the same physiological and metabolic rates when measured in the physiological time frame.

Many parameters used in pharmacokinetic modeling are directly correlated with the body weight of the particular organism. These physiological parameters generally vary with body weight according to

$$y = a\,BW^{b},$$

where y is a physiologic or metabolic parameter, and a and b are constants. If b = 1, the physiological parameter y correlates directly with body weight. If b = 0.67, the parameter y correlates with surface area.

Organ volumes tend to scale across species with the first power of body weight. Examples are total blood volume ($BW^{1.02}$) and the mass of the mammalian heart ($BW^{0.98}$). The liver is an exception, scaling with the 0.87 power of body weight. Because tissue volumes tend to extrapolate across species with the first power of body weight and biological times extrapolate with the 0.25 power of body weight, volume-based rates such as clearance rates, cardiac output, and alveolar ventilation will vary as the 0.75 power of body weight.

There is considerable evidence that cardiac output, defined as the volume of blood pumped by the left ventricle of the heart per minute, is related to metabolic rate and that metabolic rates across species are related to the 0.75 power of body weight. Ventilation of the lungs, a cyclic process of circulation and gas exchange that is basic to respiration, also scales between species. Total ventilation or "minute volume" is defined as the volume of air inhaled per minute. The fraction of minute volume available for gas exchange in the alveolar compartments is termed the alveolar ventilation rate. Minute volume and, hence, alveolar ventilation has been shown to scale across species with the 0.75 power of body weight.

Limited data are available for interspecies scaling of metabolic enzymatic activity. Cytochrome oxidase has been found to scale with the 0.75 power of body weight, and the number of mitochondria in a mammalian liver scales with the 0.72 power of body weight. Mitochondrial densities in 13 species of mammals have been shown to closely parallel maximal rates of oxygen consumption, which scale with the 0.75 power of body weight. However, more information on interspecies scaling of metabolic parameters is needed.

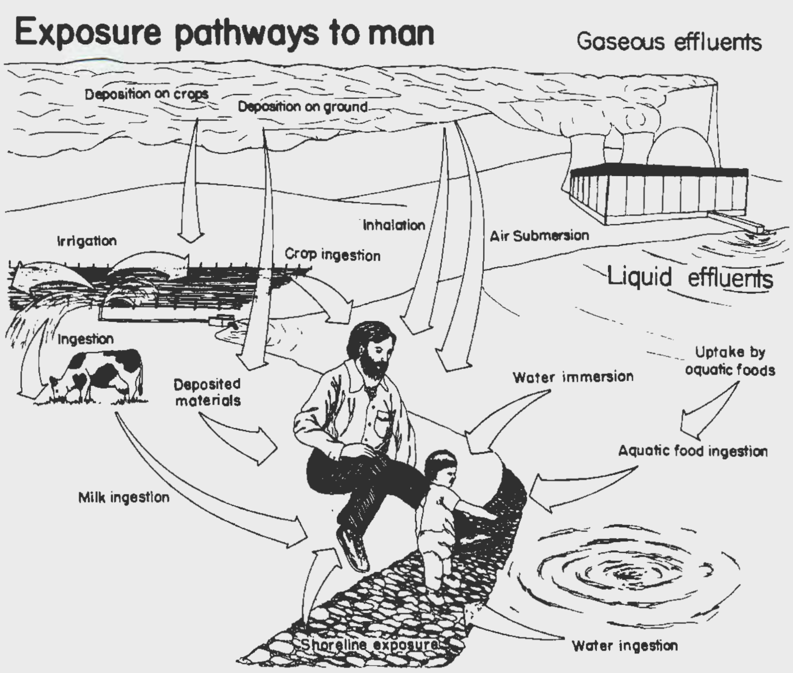
Predicting Radionuclide Transport in the Environment

By Stephen V. Kaye

Humans have always been exposed to radiation in the environment. Cosmic rays, volcanic gases, mineral deposits, and probably some radon gas in the first cave dwellings are some of the natural sources of environmental radiation exposure. But only during the last three or four decades have we humans become concerned about the health effects of radiation exposure and with learning how to track, measure, and monitor radioactivity in the environment.

In the late 1950s and early 1960s, ORNL's first ecologists conducted pioneering research in radioecology that has proven quite valuable for human health protection. They used a new tool, the radioisotopic "tag," for determining the locations and migrations of various elements in the environment. A tag is a radioactive isotope deposited in the environment as a by-product of nuclear testing or injected experimentally into components of the environment. By using a radiation detector, ecologists can trace the migration (cycling) of the "radiotracer" through the environment (e.g., by food chain transfers, decomposition, leaching, and wind dispersion) and determine its final fate. In recent years, ORNL researchers have used the technique to monitor radionuclides released from waste sites into the environment. They have collected data on radionuclide concentrations in human exposure pathways that permit realistic estimates of the dangers these pose to the surrounding human population.

The ORNL team involved in this pioneering field work was headed by Stan Auerbach and included Jerry Olson, Martin Witkamp, Dan Nelson, Dac Crossley, Paul Dunaway, and me. Our work led to a better understanding of radioactivity transfer and provided some of the first data for constructing the mathematical models now used to predict radioactivity concentrations in various "compartments" of complex aquatic and terrestrial ecosystems.



This schematic showing routes by which humans can be exposed to radioactivity was taken from Kaye's 1979 presentation to a visiting delegation of scientists from the People's Republic of China.

The Plowshare Challenge

In 1966, a challenge of international scope was presented to our group by the U.S. Atomic Energy Commission's Plowshare Project: to develop a new modeling methodology for predicting radiation doses to the public following the release and movement of radioactivity along environmental pathways. This information was needed because the AEC was considering the use of nuclear detonations to excavate large land masses (e.g., a new canal through the

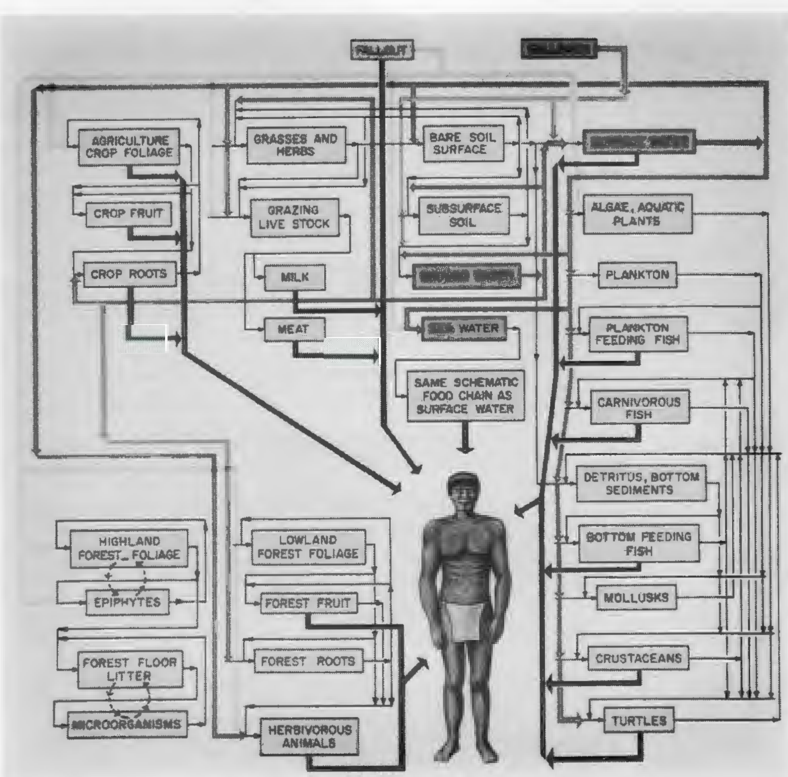
Central American Isthmus) and to stimulate the flow of natural gas from impermeable rock strata deep underground in the western United States. Using our experience with ecosystem models, ORNL ecologists and health physicists (led by Ed Struxness and Ken Cowser) responded and successfully developed a new methodology tailored to predict the potential radiological doses to humans from Plowshare applications.

Paul Rohwer (health physicist) and I were asked to develop radiation dosimetry models and compile information about the environments and native populations of remote sites in Panama and Colombia. Both countries were being considered as the site for a new sea-level canal to be excavated by nuclear explosives. Our mission was to predict how soon the evacuated people could safely return to these areas after detonation.

Realizing that we lacked the mathematical tools and a mature methodology to deal with the 18 to 21 nuclear detonations per site and the more than 200 radionuclides from each detonation, we decided to simplify the environmental radionuclide transfers, using a model of coupled compartments. The radioactivity transfers from one compartment to another were represented by rate coefficients or transfer factors. A breakthrough in our approach resulted from the interdisciplinary contacts of ORNL.

Environmental Systems Analysis

I mentioned to Syd Ball, of ORNL's Instrumentation and Controls (I&C) Division, that I had read reports indicating that reactor engineers treat different parts of reactors as compartments connected by flows of energy or materials (e.g., cooling water, heat, electrical power). I noted that the engineers' techniques for considering feedbacks and other phenomena were more advanced than those used by the environmental health physicists. Ball then briefed me on his work with Tom Kerlin at the Nuclear Engineering Department of the University of Tennessee in Knoxville, using frequency and transient analysis digital computer codes to model reactor dynamics. After further discussion, he and I saw ways of applying these reactor codes directly to analyses of radionuclide movements in ecosystems. Our preliminary work demonstrated that this dynamic modeling approach was valid and might produce results superior to those of the conventional static models. Later, I collaborated with Ray Booth, a nuclear engineer in the I&C Division, whose superb talents in systems analysis modeling greatly improved our initial environmental model. We published several papers



For Project Plowshare in the 1960s, ORNL staff developed this model predicting radiological exposure pathways to natives of Panama from a proposed nuclear excavation of a new sea-level canal.

showing that, if properly applied, environmental systems analysis is a useful tool for predicting radionuclide movements.

The NEPA Challenge

Environmental systems analysis was never used for the Plowshare program, which was terminated by the AEC about the time that a vigorous civilian nuclear power program was under way. However, this methodology became much in demand shortly after the National Environmental Policy Act (NEPA) of 1969 was enacted. The NEPA required a detailed analysis of the potential health and environmental impacts of each of the numerous nuclear facilities then proposed for construction. ORNL scientists were instrumental in developing the data bases, modeling concepts, and computer codes for implementing environmental systems analysis techniques to meet these important needs.

These and other pioneering developments in radiological assessment made by health physicists, radioecologists, engineers, and mathematicians at ORNL are still being refined in ORNL's current Health and Safety Research Division. Environmental systems analysis—as applied by ORNL, the Nuclear Regulatory Commission, the Department of Energy, the Environmental Protection Agency, and others throughout the world—is now the standard approach for predicting radionuclide transport and radiological doses to humans resulting from radioactive releases to the environment. ornl

Stephen V. Kaye is director of ORNL's Health and Safety Research Division. During the period covered by this article, he was a research ecologist in the Radiation Ecology Section of ORNL's Health Physics Division (the section was the precursor of the Environmental Sciences Division).

In this early photograph, Steve Kaye radios his location on the Oak Ridge Reservation.

Instruments for Monitoring Hazardous Chemicals

ORNL has developed instruments to monitor pollutants in synfuel facilities, indoor air, and hazardous waste sites.

By Alan Hawthorne, Richard Gammage, and Tuan Vo-Dinh

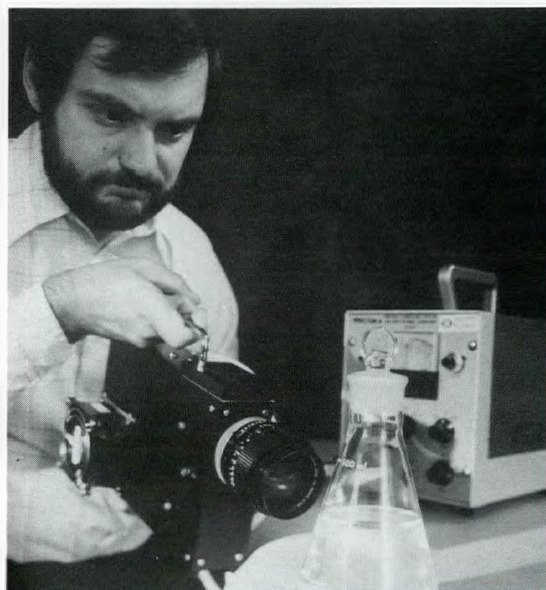
In the mid-1970s, ORNL witnessed many national-level changes that greatly affected the work of the Laboratory. The energy "crisis" began, and the AEC became ERDA and then DOE. In response to the federal requirement that the health and environmental effects of nuclear power plants be assessed, the Health and Safety Research Division (HASRD) was formed at ORNL from groups in the former Health Physics, Operations, and Environmental Sciences divisions.

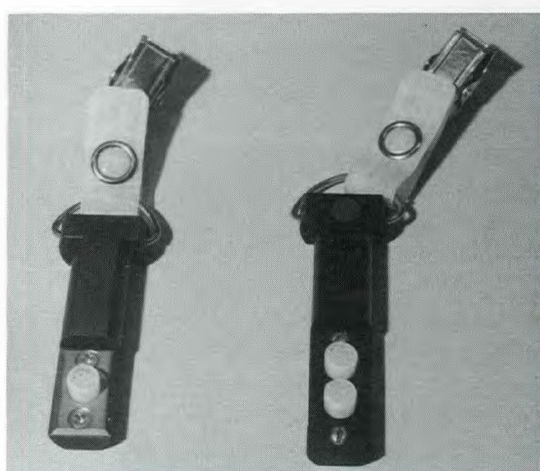
There was also a need to address health and environmental issues for nonnuclear technologies (e.g., coal gasification and liquefaction), because DOE's broadened mission included all energy sources. In response to this need, HASRD's Dick Gammage began developing methods and instrumentation for monitoring chemical pollutants and assembling a group to do this work.

Building on ORNL's experience and expertise in monitoring nuclear radiation, the group's goal was to provide a similar level of support to nonnuclear technologies. The new emphasis was reflected in the group's logo (shown here), which highlights spectroscopy and polynuclear aromatic (PNA) compounds—two prominent aspects of HASRD's research.

Luminescence Techniques

The group's initial research emphasis was on improving monitoring of pollutants from the production of synthetic fossil fuels—for both worker and environmental protection. Efforts focused on various luminescence techniques for measuring PNAs, the primary chronic hazard linked to synfuel production, because PNAs absorb ultraviolet light and then emit light (fluoresce) in the near-ultraviolet or visible regions.





The portable fluorescence spill spotter (above) and the passive PNA dosimeter are two of the award-winning monitors developed by group members.

Tuan Vo-Dinh checks Dan Schuresko for possible skin contamination at the H-Coal Pilot Plant in Cattletsburg, Kentucky.

One of the first instrumentation developments was the derivative ultraviolet absorption spectrometer (DUVAS), which identifies and quantifies specific gases and vapors in the synthetic fuel workers' environment. DUVAS works on the principle that each PNA absorbs UV light of a characteristic wavelength and fluoresces with an intensity indicating the airborne concentration of the PNA. There have been other, award-winning, instrumentation developments by members of the HASRD team. In 1980, Dan Schuresko and co-workers received an I•R 100 award for the portable fluorescence spotter instrument for PNA detection and Tuan Vo-Dinh received an I•R 100 award in 1981 for his passive PNA dosimeter. The development work on the portable fluorescence spotter was performed while Schuresko was in the Chemical Technology Division.

Another successful device developed during the synfuel years was the luminoscope, a portable fiberoptics device for measuring PNA contamination on skin and other surfaces. This instrument was developed by Vo-Dinh and built by the Instrumentation and Controls (I&C) Division. The luminoscope subsequently became the first technology licensed to an East Tennessee company through the technology transfer efforts of DOE and Martin Marietta Energy Systems, Inc. All of these instruments were field tested at operating experimental synfuel facilities such as the University of Minnesota–Duluth Gasifier and the H-Coal Pilot Plant (see photo above).

Also during this period, several advances were made in laboratory-based measurement methods. Our research on and applications of the room-temperature phosphorescence (RTP) and the synchronous luminescence (SL) methods of measuring PNAs have been widely referenced by commercial instrument manufacturers for SL and RTP applications. The group's activities during this time were an integral part of the Life Sciences Synfuel Program headed by Ken Cowser.

As the oil crisis abated and national energy policy changed in the early 1980s, there were dramatic decreases in the support for synfuel research and facilities. Many of ORNL's synfuel-related achievements in instrumentation and methods development have been successfully applied in other industries. There has been considerable interest in our luminescence techniques for rapid, cost-effective screening of environmental and work-area samples to identify hazardous chemicals.

Indoor Air Monitoring

Also during the 1980s, DOE and other federal agencies began to recognize the need to monitor indoor air quality, because buildings that were weatherized to conserve energy often had, as a consequence, reduced air infiltration and increased concentrations of indoor pollutants. These airborne toxins included cigarette smoke, formaldehyde, and radon, a naturally occurring radioactive gas that breaks down into radioactive by-products. We began research in this area because some of our existing instruments and methods were directly applicable to evaluating indoor air quality. Our challenge was to develop monitoring techniques that were capable of measuring levels lower than those detected by traditional industrial hygiene methods.

Combustion sources, including cigarette smoking, frequently produce PNAs indoors. Conventional analysis techniques for PNAs, such as gas chromatography combined with mass spectrometry, were generally too expensive, time-consuming, or inappropriate for monitoring

The fluoroimmunosensor received an I•R 100 award in 1987; development team members are (left to right) Tuan Vo-Dinh, Mike Sepaniak (UT), Guy Griffin, Kathy Ambrose, and Bruce Tromberg.

indoor air in a large number of homes. By optimizing our previously developed PNA monitoring methods, we were able to include PNAs in the list of pollutants that could be measured in indoor field studies. Our data clearly show how the indoor air levels of PNAs increase in direct proportion to the number of cigarettes smoked there.

One major challenge was to develop low-cost methods for measuring the low airborne levels of formaldehyde, which is produced by some construction and consumer products. Tom Matthews developed several methods to address this problem. The simplest of his quick-screening methods, which directly compared a chemically developed reagent with a color chart, made the cover of the *Air Pollution Control Association Journal.*

Our team also became one of the leading U.S. research groups in measuring levels of radon in homes. We are working to understand the transport between the major sources of this radioactive gas (the soil and rock underlying the house) and the interior of homes and to determine the best "mitigation measures" to reduce the indoor levels of radon and its hazardous by-products.

Biological Monitoring

We are now using ORNL instruments and new techniques to characterize hazardous waste and improve the monitoring capabilities of bioindicators. We think the use of surface-enhanced Raman spectroscopy (SERS) holds considerable promise in both areas. With support from the Laboratory Director's R&D Fund, we are developing a portable SERS fiberscope for remote monitoring of toxic organic solvents and pesticides in groundwater at parts-per-billion concentrations.

Our research on the use of biology-based monitoring is beginning to pay off. A new instrument in this area is the fiberoptics fluoroimmunosensor (FIS), which incorporates fiber optics, antibodies, and laser spectroscopy. Antibodies, which bind antigens in the sample (e.g., a body fluid), are attached to the end of an optical fiber through which a laser beam excites the antigen and the emitted light is returned for detection. FIS, which was developed by Vo-Dinh and co-workers, received an I•R 100 award in 1987. With support from the Director's R&D Fund, we plan to also contribute to the national biomedical research initiative on the human genome.

Developing instrumentation and monitoring methods to help assess the human health impacts of energy-related chemical pollutants is a major HASRD achievement. We intend to continue making important contributions to biomedical and environmental research. ornl

Alan Hawthorne, who holds a Ph.D. degree in nuclear engineering from North Carolina State University, has been at ORNL since 1976. A member of the Health and Safety Research Division, he became group leader of the Measurement Applications Group in 1982 and head of the Health Studies Section in 1987.

Field Study Motto: Be Prepared

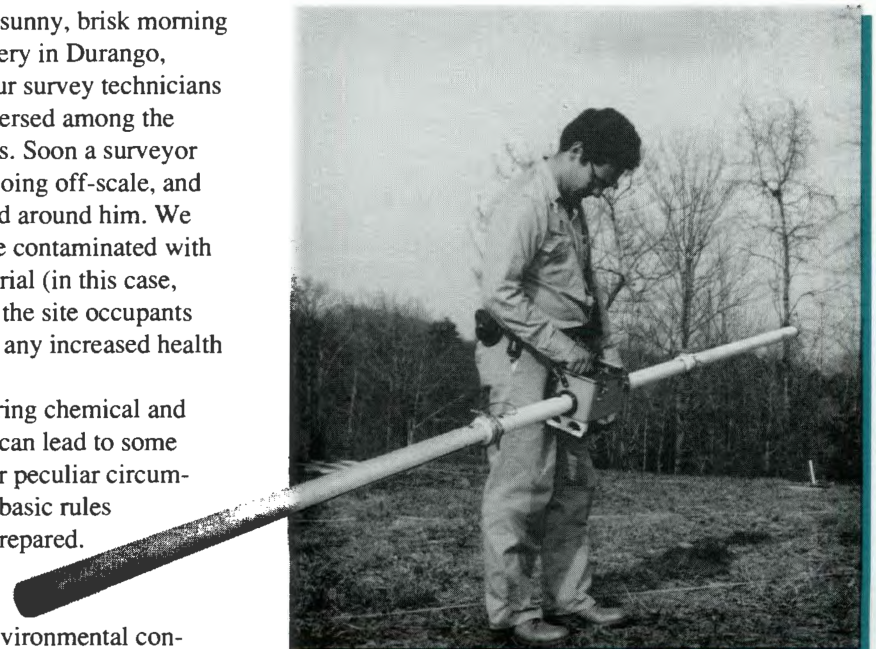
Remedial action site survey teams face bad weather, equipment breakdowns, and (sometimes) irate property owners with adaptability and good humor.

By Barry Berven and Craig Little

Arriving on a sunny, brisk morning at the cemetery in Durango, Colorado, our survey technicians quickly dispersed among the headstones and monuments. Soon a surveyor yelled that his meter was going off-scale, and the team quickly assembled around him. We had discovered another site contaminated with low-level radioactive material (in this case, uranium mill tailings), but the site occupants here would not experience any increased health risk.

Our field studies measuring chemical and radiological contaminants can lead to some unusual places, often under peculiar circumstances; hence, one of our basic rules is to be flexible and well prepared.

ORNL has historically been the lead national laboratory for assessing environmental contaminants and their impact on human health in the United States. During the past ten years, much of our effort has focused on the Department of Energy's remedial action programs, such as the Uranium Mill Tailings Remedial Action Project (UMTRAP), Formerly Utilized Sites Remedial Action Project (FUSRAP), and the Surplus Facilities Management Project (SFMP). Recently, major new initiatives have begun in characterization and remediation of chemical contaminants at DOE and Department of Defense (DOD) sites. ORNL's Health and Safety Research Division (HASRD) has developed capabilities to identify and quantify virtually any radiological or chemical contaminant, using state-of-the-art field survey techniques. The survey team in the Durango cemetery was a part of this effort.



A member of a survey team measures subsurface conductivity to locate buried metal waste drums.

Before any work begins, the first question is always "What is the survey's purpose?" Exploratory surveys determine if contaminants are present, and site-characterization surveys determine the magnitude and extent of contamination. Sometimes we are trying to confirm the *absence* of contaminants (e.g., to determine the adequacy of remediative measures).

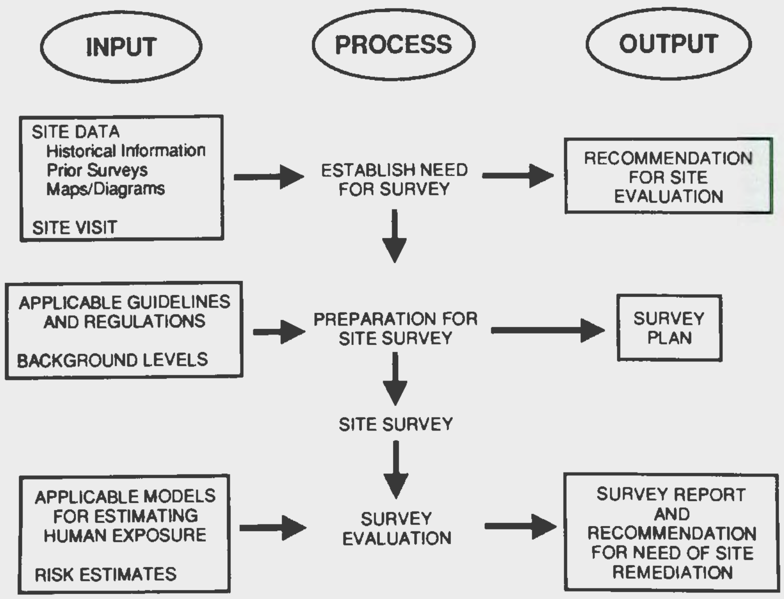
The first step in any field investigation is to gather as much historical information about the site as possible (see figure). Knowing the processes and materials used at a site often identifies the most likely contaminants and their location. Although helpful, information from earlier surveys may be inadequate and must be used cautiously.

A Common-Sense Approach

Field investigations are discovery processes; usually the contaminant, its location, and the quantity are unknown. Like a good book, although the end is satisfying, the doing is all the fun, and common sense is a field investigator's greatest asset.

Planning Pays

The single most important aspect of these field investigations is the survey plan outlining the work to be performed. The data we gather in a survey from instrument measurements and sample analysis must have an accuracy and confidence



Field surveys are well-planned discovery processes.

level that will satisfy guidelines and regulatory limits for the contaminants we are interested in finding. The type of instrumentation used, the amount of in situ monitoring vs destructive sampling, and laboratory procedures must be selected to ensure that the accuracy level can be met. Often guidelines or regulations are expressed as concentration limits (maximum acceptable value) of contaminants in various media (e.g., picocurie of radionuclide per gram of soil, parts of contaminant per million parts of water, etc.). Standards are sometimes set at levels below the reliability of portable monitoring instruments, especially for most chemical contaminants. In these cases, we rely on collecting environmental samples that are processed in analytical laboratories.

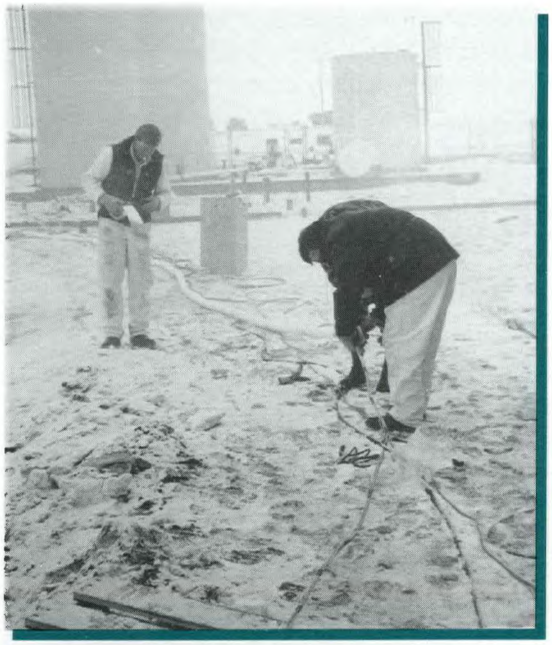
In the planning process, it is also important to know the naturally occurring background concentration of a contaminant. Knowing the ambient level will help the surveyor select instruments or sampling processes that can adequately measure the naturally occurring levels and will enable the surveyor to correct his contaminant measurements by subtracting the background values.

Once the contaminant of concern is identified, we must investigate its pathways for potential human exposure. These are often specified by guidelines or regulations. For example, if guidelines limit contaminant concentrations in terms of picocuries per gram of soil, then soil concentration becomes the primary focus of the survey. Several exposure pathways may be examined, such as inhalation (measurements of air-suspended contamination) or ingestion (measurements of contaminants in water, vegetables, milk, or meat). Instrumentation and sampling protocols are selected to suit the pathways being investigated.

Planning must also include measures to ensure the health and safety of survey personnel and to maintain quality assurance and quality control. Safety of survey personnel is always foremost. Our staff members are trained to understand the actions and protective gear required in contaminated (possibly lethal) environments and the pathways of potential exposure and health risks from various levels of these exposures. Because most sites have only low levels of contamination, our primary focus is on measures to prevent chronic exposures, but unexpectedly high levels may be found. Team members must be trained to handle these rare potentially life-threatening situations.

If we cannot document that we have performed a survey in a reliable and reproducible manner, the survey team's credibility is lost, and the value of the work is questionable. ORNL surveyors take pride in the quality of their careful documentation of procedures, survey activities, and analytical results. Quality control is maintained by frequent review and independent audits of these activities.

Cost estimation and the commitment of resources required to perform the survey constitute the final aspect of planning. Resources will include staff, equipment, materials, supplies, travel, analytical needs, and documentation and reporting costs. We must also make a realistic estimate of the time needed to perform the survey and report the results.



Survey teams learn to expect the unexpected, such as this sudden change in the weather at an Air Force base in Utah.

Expect the Unexpected

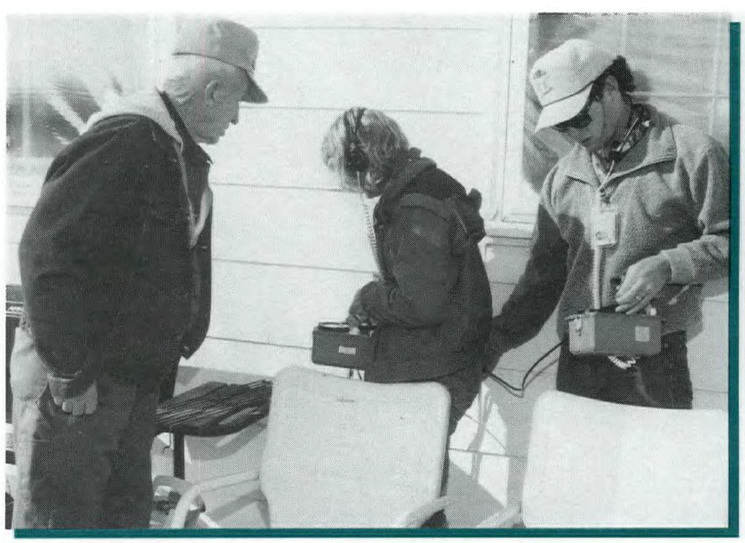
Once the survey is well planned, the rest is easy, right? Wrong. Executing the plan is where theory meets reality. Picture yourself arriving on a survey site with a crew of dedicated, well-trained technicians and a handful of survey plans—only to find yourself in the middle of a snake-infested desert in Arizona, a slum in New Jersey, or a Florida swamp during a torrential downpour!

At any survey site, the first step is to meet with local officials, property owners, environmental agencies, and, occasionally, the media to introduce our organization and explain what we intend to accomplish. We try to assure the community of our commitment to performing a high-quality survey. Next, the survey team must establish or review a previously established grid system defining the area to be surveyed. We use this to select locations where measurements and samples are taken and to relocate areas of concern, if necessary.

Once the grid has been established, the survey plan is carried out. This may mean drilling groundwater monitoring wells to sample for chemical contaminants at an Air Force base in Texas, collecting aquatic biota (pond slime and fish) to measure radionuclide contaminant levels in effluent from a nuclear power plant, or monitoring and sampling volatile organic compounds in soil from a fuel spill at a DOE facility.

The survey itself is the most exciting part of this business. Rarely does it go according to plan; unexpected circumstances call for creative problem solving in the field. Sometimes the complications experienced are ordinary: a survey is carefully planned, six months in advance. The materials, instruments, supplies, and staff all arrive on a perfectly executed time schedule. The survey team arrives on-site, and suddenly the worst weather in recent history occurs! The mud in the field is so sloppy or the snow so deep that the team can't even walk, much less drive a drill rig or survey vehicle on-site. The only consolation comes from local residents, who report that last week was worse.

Problems often arise just because of the nature of our work. The purpose of the survey is to find contamination, and a frequent surprise is finding



Interested homeowners sometimes participate in our surveys.

more or less of a contaminant than we expected or finding it in an unanticipated spot. Having set up on a site, the team discovers that the contamination is a half mile down the road; faulty historical records or contaminant migration are to blame.

Owners of the properties we survey present their own challenges. Survey teams have on occasion been met by property owners who invited them in for tea and by others who brandished shotguns. Surveys have also been performed late at night to accommodate commercial owners concerned about public relations. Restaurant proprietors seem to be particularly sensitive.

One of the more unusual problems we've encountered was at a site near the Oak Ridge Y-12 Plant. We were completing a ground-surface survey for possible depleted uranium deposits near some office trailers, when one of our technicians observed an increased radiation level near the sidewalk. After following it for a short distance, he noticed that the level dropped. A short distance farther down the sidewalk, the level increased again. Standing still for a moment, he saw the level again decrease. Clearly, a radiation source was present but apparently it was moving! Another technician resurveying a nearby area that was earlier found clear of any contamination suddenly observed elevated radiation levels. After some head scratching, the survey technicians tracked down the source of the radioactive material—a man inside one of the trailers who had received radioactive iodine earlier in the week during a thyroid function test. Within a few days, the iodine decayed and the man was no longer a source. (Interestingly, the man's secretary was advised to move to a separate office during the intervening period.)

Surveys have inherent human-error problems such as mistakes in sample labeling and failure to collect enough samples or make enough measurements. It is difficult to resurrect missing data, samples, or measurements from a survey conducted 1000 miles away once you are back at ORNL. Survey teams also run out of supplies, wish they had packed a different instrument, or have to readjust their time schedules sometimes because of unforeseen situations.

Subsurface drilling is sometimes required for sample collection, as at this jet fuel spill site.

Reporting Results

At the end of the survey, it's time to think about how to explain the survey findings to the sponsoring agency. The purpose of the survey report is to clearly and succinctly explain the results and significance of the field investigation. This is done by clearly defining the purpose of the investigation, relating some of the site history, and presenting the data in well-organized tables and figures. The easiest situations to explain are those in which the site is either grossly contaminated or clear of all contaminants. Conversely, the most difficult situations to explain are those where contaminant levels are close to the guidelines. Such marginal contamination requires additional samples and measurements to determine the need for remediation. Thousands of taxpayer dollars are in the balance.

Interpretation of survey data is often difficult because of conflicting and confusing information. For example, while a survey team was on a site in Kansas, the contaminant trichloroethylene (TCE) was found in the groundwater. In attempting to determine where it was going and how fast, scientists placed and sampled monitoring wells several hundred yards down gradient from the primary contaminant area. Very little TCE was found, but a lot of vinyl chloride was present, a fact not indicated by the historical record. After some investigation, it became clear that microbial agents were degrading the TCE into vinyl chloride through several intermediate stages.

On another site in New Jersey, the survey focused on radioactive thorium, a by-product from processing monozite sands for rare-earth elements. No significant levels of thorium were found, but high levels of radium contamination from another area had migrated onto the site under investigation. Detailed chemical analysis demonstrated that the contaminant was unrelated to government-contracted activities and would have to be considered for cleanup under remedial action programs other than those we represented.

A part of every survey report is a risk analysis to determine potential exposures and risks to humans. This can be as straightforward as determining the direct gamma radiation exposure to a person frequenting a contaminated site or as complex as modeling the transport of a chemical contaminant through its physical and chemical changes in a subterranean environment. All potential pathways for human exposure are examined, and the most realistic for the particular site are targeted. Models using computer codes are frequently employed to simulate the movement of contaminants through water, soil, and/or air. Using our survey measurements, the models can derive estimates of chronic and acute exposure to human occupants of the site. Estimated exposures are either compared with existing guidelines, or a risk assessment is done. Often the risk to humans from various chemical and physical agents is poorly understood, or the assessment has been based on exposure to a single agent, while a multitude of chemicals having compounding effects may be present at the site. Most available risk data are based on animal studies that may or may not be applicable to humans.

During the reporting process, we realize the value of a well-conceived survey plan and an excellent quality assurance program in defending the conclusions and recommendations for remediative measures at a site. A carefully planned and executed survey deserves a well-written, defensible report. Most people involved in making remediation decisions do not visit the sites and never see the effort put forth in site preparation and sample collection or the care used in taking measurements among the weeds, dirt, and muck. Decisions on the disposition of a site are based on the data and their presentation in the report and on the defensibility of the interpretation used to reach conclusions and recommendations.

Investigating radiological and chemical contaminants at a site is a complex process that requires a multi-disciplinary approach from a close-working team of professionals.

Investigating radiological and chemical contaminants at a site is a complex process that requires a multidisiplinary approach from a close-working team of professionals. Staff training and education are important requirements, but dedication and experience are equally necessary. As in most endeavors, the survey and assessment are only as good as the people performing the work.

As for the Durango cemetery, because we believed the current site occupants were at no additional risk from exposure to uranium mill tailings, the ORNL team recommended to DOE that no remedial action be taken, and our survey, like the site occupants, was laid to rest. ornl

Barry Berven is head of the Environmental Measurements and Applications Section of ORNL's Health and Safety Research Division. He came to ORNL in 1979 after receiving a Ph.D. degree in health physics from Colorado State University.

Craig Little is leader of HASRD's Pollutant Assessments Group and manager of ORNL's Grand Junction Office in Colorado. He came to ORNL in 1976 after obtaining his Ph.D. degree in radioecology from Colorado State University.

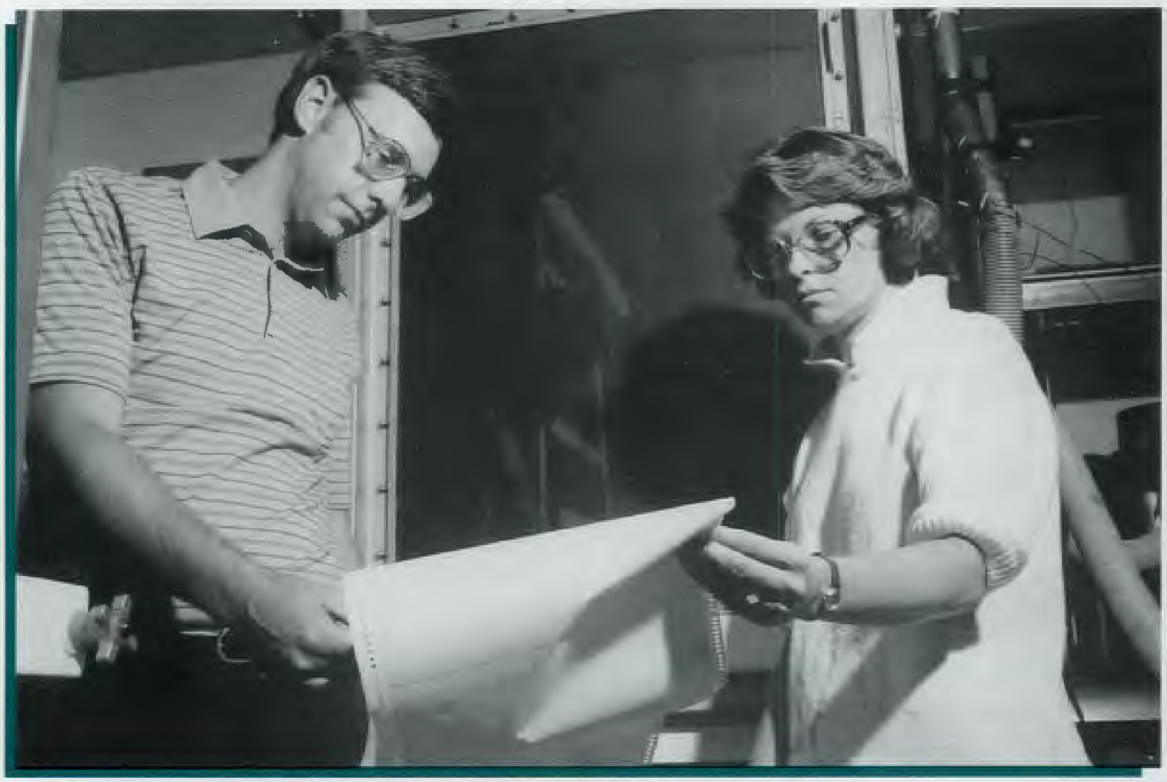
Information Research and Analysis

By Tim Ensminger

The end product of scientific research is new data and information. A wealth of scientific information resources and data management capabilities has been developed in association with ORNL's research and development activities. Many of these resources are managed by the Information Research and Analysis Section (IR&A) of the Health and Safety Research Division (HASRD).

Since the late 1960s, IR&A staff have performed data analyses and information management in three major research areas: health and environmental effects of hazardous substances, energy technology development, and waste management/remedial actions for contaminated lands and facilities. The work takes many forms: development of computer data bases and data systems; validation of existing data sets; production of bibliographies and chemical profiles and in-depth assessment reports. The assessments cover a variety of topics ranging from potential effects of hazardous materials to studies of research and regulatory methodologies. As a spin-off of extensive efforts in developing data bases and data systems, the section has also become involved in studies of new and better methods for information management and dissemination.

Reliable data form the basis for sound judgment in any scientific arena. Accurate information is essential in identifying and preventing adverse health effects linked to exposures to toxic substances. These substances include the steadily increasing list of chemicals being used today for agricultural and industrial purposes as well as the toxic chemicals that are migrating through the soil from forgotten waste disposal sites into groundwater supplies.



Bob Ross (HASRD) and Roswitha Ramsey (Analytical Chemistry Division) are designing new methods for exposing test animals to combustion products from weapons discharges.

ORNL serves several federal agencies by acquiring and analyzing data on health effects of toxic chemicals.

Rose Haas is developing a computerized system to help identify chemical substructures having the potential for causing genotoxic effects.

In most cases, data on the effects of toxic chemicals on humans are nonexistent. Identification and prevention of adverse health effects must, in large part, be based on data obtained from animal testing. Such extrapolations are obviously inexact and create the potential for unknown levels of error. Thus, the data on which such estimates are based must be the best available.

Chemical Toxicity Data Bases

The IR&A Section has long been committed to developing high-quality data bases and assessments for health effects. Since 1974, IR&A staff have been developing the Hazardous Substances Data Bank (HSDB) as a comprehensive chemical effects information resource. It has set the standard for toxicological data bases. Each of the 140 data fields on each chemical is reviewed by a panel of experts that meets quarterly. The data base, which currently contains information on about 4200 chemicals, is accessible through the TOXNET system of the National Library of Medicine.

In 1979, IR&A staff became involved in the Gene-Tox Program of the Environmental Protection Agency (EPA). This intensive effort in data validation by 23 panels of experts has depended heavily on the data resources of the IR&A Environmental Mutagen Information Center. The purpose of the Gene-Tox Program is to develop a validated data base from the results of 73 genetic toxicology bioassays (tests indicating the genetic effects of certain toxins on specific organisms). Continuing assessment of the data will assist EPA in identifying the most efficient and cost-effective batteries of tests for genotoxic effects. It will also help in developing future research directions and in identifying potential adverse health effects of several classes of related chemicals. Validated genetic toxicology data are now available for 4000 chemicals.

The HSDB and Gene-Tox are used as resources by IR&A staff in preparing assessment reports for EPA, the Department of Defense, and several state governments as well as in the development of a Materials Safety Data Sheet (MSDS) Data Base for Martin Marietta Energy Systems, Inc. The MSDS data base is used to notify employees of the Oak Ridge, Paducah, and Portsmouth facilities of potentially hazardous materials in the workplace. Hazard notification is a requirement of the Occupational Safety and Health Administration Hazard Communication Standard of 1980. Energy Systems, however, has elected to comply with the spirit as well as the letter of the law by using our validated data to complete and enhance information received from chemical manufacturers.

The emergence of the IR&A Section at ORNL as a national center for high-quality toxicology data resources and data assessment has added a vital component to the HASRD's strong capabilities in hazard assessment and exposure-dose modeling.

Remediation Support

Measurement and remediation are key terms in the current era of seemingly endless disclosures about contaminated sites and facilities. With the passage and continuing revision of environmental legislation such as the Resource Conservation and Recovery Act (RCRA); Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA); Toxic Substances Control Act (TSCA); Clean Air Act (CAA); Superfund, Amendments, and Reauthorization Act (SARA); and Clean Water Act (CWA), regulatory pressure has intensified for industry and for government agencies that were previously exempt, for national security reasons, from liability for environmental pollution. The Department of Energy (DOE) and the Department of Defense (DOD) are two such agencies. Today, both agencies have extensive programs under way to identify and clean up sites and facilities contaminated with either radioactive or chemical pollutants.

Work for DOE

An Information Management System (IMS) is being developed in support of DOE's Human Genome Initiative by the IR&A Section. This information system will serve as the primary means of facilitating communication and information exchange among researchers, contractors, and grantees working on DOE's Human Genome Initiative. The IMS will also serve as an information outlet to other scientists interested in keeping abreast of developments regarding research on the human genome. During the current fiscal year, three tasks have been identified for implementation as the first part of the overall IMS development. These are: (1) publishing a quarterly newsletter, (2) setting up an electronic bulletin board, and (3) publishing two technical reports.

The IR&A Section also has been actively involved in work related to identification and remediation of environmental contamination since the early 1970s. Our initial efforts helped develop a transuranic elements data base for the DOE Nevada Applied Ecology Group (NAEG) at the Nevada Test Site. Since the completion of the NAEG mission, we have focused on (1) assessing data on the worldwide fallout of plutonium to determine reliable background levels and (2) furnishing support to the DOE and ORNL Remedial Action Programs.

The IR&A Remedial Action Program Information Center provides information on the scientific, technological, regulatory, and economic aspects of cleaning up contaminated DOE sites and facilities. The Center supports all five subcomponents of the Remedial Action Program (RAP): Surplus Facilities, Formerly Utilized Sites, Uranium Mill Tailings, Grand Junction, and the Technical Measurements Center. On the local level, the Center assists ORNL's remedial actions through the development of internal data bases.

IR&A staff have also collaborated extensively with the Environmental Measurements and Applications Section within HASRD in measuring levels of contamination, analyzing the data, and recommending remedial actions at sites associated with the five RAP subcomponents as well as the

Oak Ridge facilities. Additionally, we have provided assistance in developing a computer-assisted program to allow data displays along with diagrams of the facilities.

We also developed a comprehensive information system for DOE's Fossil Energy Program (FEP) to track both management and technical data over the life of the program. Through close collaboration with FEP researchers and administrators, the IR&A staff developed the Fossil Energy Environment, Health, and Safety Information System to capture emissions data at plant sites and, thereby, document any instances in which legal environmental limits are exceeded. The system will also be of value to researchers in DOE's Clean Coal Program who assess environmental releases and develop technologies for reducing them.

Work for DOD

DOD environmental concerns range from individual exposure to toxic materials in the line of duty to environmental effects of waste streams from munitions production plants. The exposure of individuals to hazardous substances has been studied by IR&A staff principally in the context of confined spaces such as bunkers and tank compartments. Data on chemical concentrations and biological effects, principally in animals, are evaluated and analyzed at ORNL to provide the best prediction of potential adverse health effects on humans. In a project undertaken in collaboration with the Analytical Chemistry Division, we are studying improved methods of exposing test animals to combustion products from weapons discharges.

Environmental work for DOD has consisted of analyzing data on the concentrations of chemicals in waste streams to determine potential routes and levels of exposure for living organisms. These data are then compared with available information on adverse biological effects to quantify potential problems and determine the extent of remediation required.

In summary, the problems of hazardous substances in the environment are long-term and of national importance. The data acquisition, evaluation, and analysis capabilities of the IR&A staff will continue to be available to concerned government agencies to help them make informed decisions. ornl

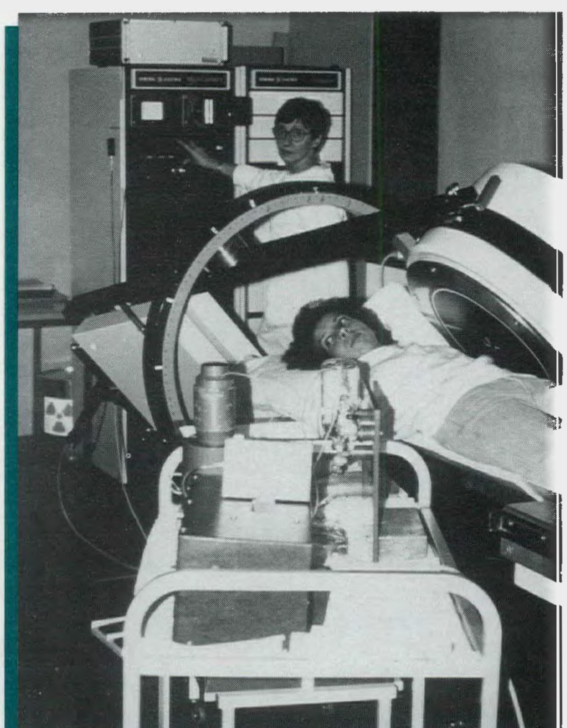
J. Tim Ensminger, was formerly head of the Information Research and Analysis Section of the Health and Safety Research Division, the ORNL Data Management Coordinator, ORNL's representative to the Energy Systems Information Resource Managers Committee, and ORNL's liaison to the Numerical Data Advisory Board of the National Research Council. He began work at ORNL in 1975; he has recently moved to the Energy Division's Integrated Analysis and Assessment Section.

Nuclear Medicine Research at ORNL

By Russ Knapp, Kathleen Ambrose, Al Callahan, Dan McPherson, and Prem Srivastava

From the earliest days at ORNL, an important peaceful application of nuclear technology has been the production of radionuclides for biomedical research and the development and distribution of radiolabeled substances for clinical diagnosis and therapy. In fact, the Atomic Energy Act of 1949 specifically earmarked funding for these nuclear medicine applications. The first nuclear medicine procedures, developed in the 1940s and 1950s, used radioactive iodine to evaluate thyroid function. From this simple beginning has evolved a wide variety of procedures that use complex "radiopharmaceuticals" to diagnose and evaluate diseases of the heart, brain, and other major organs.

In biomedical research, radioisotopes serve as unique and powerful tools to monitor the biochemical transformation of natural body components. Radiolabeled compounds are also widely used to evaluate the metabolism of drugs, pollutants, and other substances and to monitor both organ anatomy and function. Following the inhalation or intravenous administration of a radiopharmaceutical, it is distributed by the body's circulatory system. The isotope-carrying molecule must be carefully chosen, and sometimes specially designed, so that it will have a particular affinity for the organ or body system that is of interest to the physician. When this is true, the isotope will concentrate in that area sufficiently to be detected with a "gamma camera" or other imaging device (see photo). Because the use of radiopharmaceuticals is noninvasive and the dosage required to produce an image is extremely low, the technique poses little discomfort or danger to the patient.



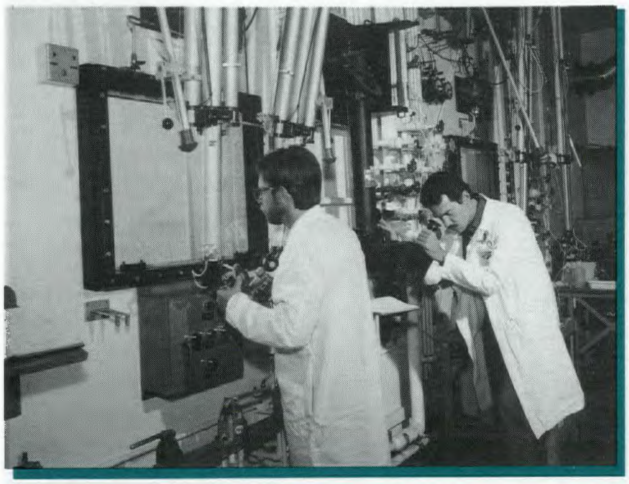
The osmium-191/iridium-191*m* generator system in the foreground was developed at ORNL and is being used in patient studies in conjunction with investigators from the Cyclotron Research Centers, University of Leige, Belgium, to diagnose and evaluate diseases of the heart.

ORNL's current Nuclear Medicine Group was formed in 1975, after the Biomedical Radioisotope Program was transferred from the Isotopes Division to the Operations Division. In 1977, the group was moved to the newly formed Health and Safety Research Division. Our radiopharmaceutical design work began when an external committee of nuclear medicine experts advised ORNL to supplement its medical isotopes production work with radiochemical design and development research, including animal testing of the designed reagents. The advisers also recommended that ORNL involve other research organizations in the testing of new medical imaging agents developed at ORNL. These goals are being met today in the work of the Nuclear Medicine Group.

New Radiolabeling Techniques

During the past ten years, our group has focused primarily on the development of new radiopharmaceuticals for imaging the heart and brain and for tumor detection. A development that has generated widespread interest and recognition in the research, clinical, and commercial sectors is the concept of "metabolic trapping" of radioiodinated fatty acids for heart imaging (see *ORNL Review*, No. 4, 1984). Our ORNL agent developed using this concept is being further developed by Japan's

ORNL's program has produced new agents for diagnosing heart and brain disease and locating tumors.



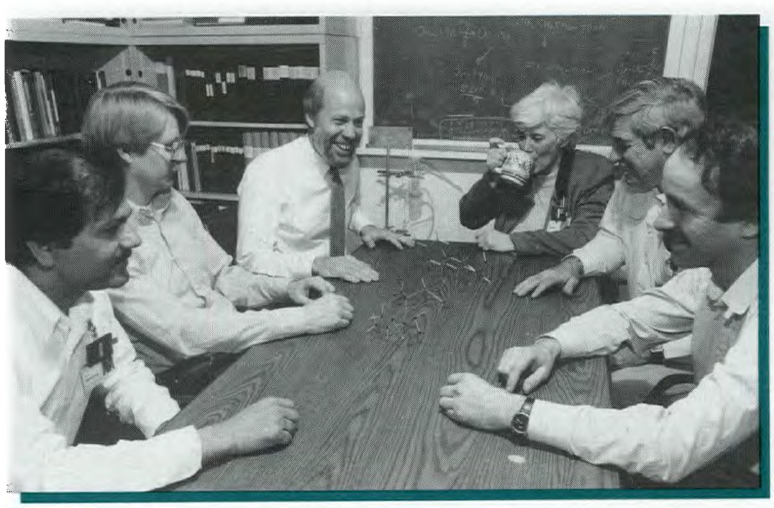
Dennis Rice, left, and John Allred process radioiostopes in hot cells for use in radiopharmaceutical research.

principal radiopharmaceutical company, Nihon-Medi Physics, for cardiology applications. Mallinckrodt, a U.S. radiopharmaceutical manufacturer, has developed our concept further to make its own imaging agent.

In 1983, we began the development of a new osmium-191/iridium-191*m* radionuclide generator system, which provides a high yield of iridium-191*m* for clinical use. This isotope has a 4.9-s half-life, which is in the ideal time range for its use in cardiac studies. The new system has been used successfully in evaluating heart performance in more than 600 patients at European hospitals. We have applied for approval to begin using the agent for U.S. patient studies at a California hospital later this year (see *ORNL Review*, No. 1, 1988). Martin Marietta Energy Systems, Inc., is currently negotiating with a major U.S. radiopharmaceutical company interested in manufacturing the new ORNL generator system.

More recent projects of our Nuclear Medicine Group include a new maleimide reagent for the radioiodination of antibodies. The use of radiolabeled antibodies for both the detection and treatment of tumors has grown rapidly since it was recognized that tumors produce surface antigens and that radiolabeled antibodies could be used to seek out those antigens. The ORNL-developed maleimide compound makes it easier to attach stabilized radioiodine groups to protein molecules of the cancer-seeking antibodies that have both therapeutic and diagnostic applications (see *ORNL Review*, No. 1, 1988). The stabilized maleimide form of radioiodine also remains attached more securely to the antibody than do radioiodine compounds in other reagents. Thus, the radioactive isotope will remain at the tumor site, rather than detaching from the reagent molecule and exposing the patient's healthy tissues—a major problem and health hazard associated with most other radioiodination techniques. Two patents have been granted for this technology, a patent waiver is in place, and Martin Marietta Energy Systems, Inc., has signed a licensing agreement with E. I. du Pont de Nemours & Company.

In developing therapeutic agents for treatment of cancer, we have recently developed a new tungsten-188/rhenium-188 radionuclide generator system. The rhenium-188 produced by this system is useful for antibody radiolabeling and other therapeutic applications, such as treating bone



Russ Knapp, (left center) heads ORNL's Nuclear Medicine Group, which includes (from left) P.C. Srivastava, Dan McPherson, Knapp, Kathleen Ambrose, Al Callahan, and Joachim Kropp.

Kathleen Ambrose, left, and Carla Rogers use rats to test the metabolism of new radiopharmaceuticals.

cancer. Rhenium-188 is a desirable radioisotope for these applications because it is readily available from our generator, has versatile chemistry for attachment to antibodies, and has a sufficiently long half-life (17 h) to allow uptake of the antibody by the tumor before imaging. In the area of protein radiolabeling, we are interested in developing improved bifunctional chelating agents for attaching radioisotopes of copper and rhenium. The rhenium-188 from our generator will be used for this purpose. A more complex approach is required, because we are introducing two chemically reactive groups on the same molecule. The first group strongly binds the rhenium or copper radioisotope to the chelating molecule, and the second binds the chelate to the appropriate protein polymer. This new project is in the early stages of chemical development.

Another new investigation by our group focuses on developing a radio-labeled nucleoside for imaging tumors. Nucleosides are important constituents of the nucleic acids involved in cell division. Because cell division is more rapid in tumors than in normal tissues, they concentrate higher amounts of nucleosides. If we can find a way to enhance the tumor uptake of radio-labeled nucleosides, visualization of solid tumors may be possible.

Brain Studies

Nuclear medicine procedures currently play an important role in detecting abnormalities of the central nervous system. Several radioiodine-labeled drugs have proven useful for diagnosing and managing neurological disease. We have begun work in this area and expect to spend considerable effort developing iodine-123–labeled drugs that bind to specific "receptors," or cellular regions, in the brain. These special molecules will be designed to bind strongly to brain receptors, just as the brain's natural neurotransmitters (chemical compounds that promote the transmission of nerve impulses through the brain) bind to particular sites. Many neurological diseases are characterized by either an excess or deficiency of these neurotransmitters. For example, Parkinson's disease, characterized by the loss of muscular control, is caused by a deficiency of the neurotransmitter dopamine. In contrast, the unpredictable behavior of schizophrenics appears to be associated with excessively high levels of dopamine.

We hope that the use of iodine-123–labeled analogs of neurotransmitters using single-photon-emission computerized tomography (SPECT) will give researchers a unique means of noninvasively mapping the endogenous locations and levels of these biochemicals. Aberrations in the endogenous levels of these neurotransmitters are known to occur in many major functional and psychological disorders.

Several developments of our Nuclear Medicine Group, such as radiolabeled fatty acids and the osmium-191/iridium-191*m* generator, are being used in clinics both in the United States and abroad. In addition, some of these agents are being considered by industry as strong candidates for further development, marketing, and clinical use. A prime example is the methyl-branched fatty acids for the evaluation of heart disease. This agent has evolved from an ORNL concept, through our chemical development research, radiochemistry, animal screening, and more sophisticated animal model studies to become a useful radiopharmaceutical for evaluating human disease. We are continuing to study fatty acids at ORNL, focusing on the cellular interactions at the molecular level, including metabolism of these compounds.

Before ORNL's new radiopharmaceutical agents can be used clinically, however, they must be evaluated in sophisticated animal model systems developed elsewhere through our collaborations with outside investigators. Through the medical cooperative programs, we can also test these agents in clinical studies, combining our radiochemistry expertise with the knowledge and experience of medical investigators in fields such as cardiology, oncology, and immunology. Several European investigators have provided us an opportunity to initiate clinical studies using our new imaging agents. More recently, we have initiated joint programs with the Cardiology Department of Crawford Long Hospital, at Emory University in Atlanta, and with the Nuclear Medicine Division of the University of Tennessee Research Hospital in Knoxville.

ORNL's Nuclear Medicine Program has enjoyed strong support from the Department of Energy in designing and developing improved radiopharmaceuticals. We expect to continue this work, while also seeking opportunities to move into new areas of interest. ornl

Russ Knapp is leader of the Nuclear Medicine Group of ORNL's Health and Safety Research Division. He received a Ph.D. degree in biochemistry from St. Louis University School of Medicine and came to ORNL in 1975, after working as a research biochemist at Rice University in Houston. He is recipient of a HASRD Technical Achievement Award and a Martin Marietta Energy Systems Inventor Award. He is editor of the book *Radionuclide Generators—New Systems for Nuclear Medicine Applications*.

Kathleen Ambrose, who received her M.S. degree in microbiology from the University of Tennessee, has been a staff member in the Nuclear Medicine Group since 1976. She was corecipient of an I•R 100 Award, a Martin Marietta Energy Systems Significant Event Award, and an Energy Systems Publication Award.

Al Callahan, a member of the Nuclear Medicine Group since its inception, has 35 years of experience in isotope enrichment, reactor operation, radiochemistry, and gamma and X-ray spectroscopy. He was one of the last operators at the Graphite Reactor before its decommissioning in 1963.

Dan McPherson, a research staff member of the Nuclear Medicine Group, did postdoctoral studies at Brookhaven National Laboratory and worked at Nova Pharmaceutical Corporation, before joining ORNL in March 1988. He received his Ph.D. degree in organic chemistry from Auburn University.

P. C. Srivastava, who received his Ph.D. degree in organic-medicinal chemistry from the Central Drug Research Institute, Lucknow University, India, has been a Nuclear Medicine Group staff member since 1981. He previously worked as head of pharmaceutical chemistry at ICN Pharmaceuticals in Irvine, California. He is recipient of the National Research Service Award for Thrombosis Research, a Martin Marietta Energy Systems Inventors Award, and a United Nations Development Distinguished Scientist Award.

RE: Awards & Appointments

Murray Rosenthal

William Fulkerson

Truman Anderson

Robert Shelton

ORNL Director **Alvin Trivelpiece** has named **Murray W. Rosenthal** deputy director of ORNL. **William Fulkerson** has been appointed acting associate director for Advanced Energy Systems, replacing Rosenthal, and **Truman Anderson** has become director of Planning and Management in the Director's Office. **Robert Shelton** has been named acting director of the Energy Division, replacing Fulkerson.

Robert I. Van Hook has been named coordinator of ORNL's Global Environmental Studies Program.

James B. Roberto has been named associate director of ORNL's Solid State Division.

The National Science Foundation has appointed **Bruce L. Kimmel** a member of the Advisory Panel for Ecosystem Studies.

Michael J. Sale received a letter of commendation from the administrator of the Environmental Protection Agency for his contributions in data base design and management for the National Surface Water Survey.

Robert J. Luxmoore has been elected a Fellow of the Soil Science Society. He also has been named to a Ground Water Recharge Committee of the National Research Council's Water Science and Technology Board.

S. Marshall Adams has been named a Fellow of the American Institute of Fishery Research Biologists.

Allen Ekkebus has joined ORNL's Office of Planning and Management, where he will provide staff assistance to the Office of the Laboratory Director.

James K. Bryson has been named director of ORNL's Personnel Division, replacing **JoEllen Meredith**, who is now director of Compensation for Martin Marietta Energy Systems, Inc.

ORNL scientists who received awards from the East Tennessee Chapter of the Association for Women in Science are **Nancy Dudney,** for distinguished and sustained contributions to science; **Carol Oen,** for being a distinguished leader and advocate and for her outstanding service to the local chapter; and **Audrey Stevens,** for distinguished and sustained contributions to science.

Po-Yung Lu has been named head of the Information Research and Analysis Section of ORNL's Health and Safety Research Division, replacing **Tim Ensminger**, who has accepted a position with the Energy Division.

G. Ray Satchler has been granted a Doctor of Science degree by the University of Oxford in England.

Donald L. DeAngelis has been named to the editorial board of the journal, *Mathematical Biosciences.*

Barry Burks has been named director of the new centralized Office of Guest and User Interactions at ORNL.

Carolyn Krause, Cheryl Koski, and **LaWanda Klobe** were elected vice president, secretary, and treasurer, respectively, of the East Tennessee Chapter of the international Society for Technical Communication. In 1989 the chapter received STC's Chapter Achievement Award for chapters having less than 200 members and the Outstanding Performance Award for STC Chapter Public Relations.

Martin Marietta Energy Systems, Inc., honored its employees on May 19, 1989, at the fifth annual dinner and awards presentation at the Hyatt Regency Hotel in Knoxville. Specially honored ORNL employees were **Claudette G. McKamey,** Inventor of the Year (cited for her "work in the development of a series of iron aluminide alloys having improved properties for structural applications"); **Salil K. Niyogi,** Scientist of the Year (cited for his "development of significant structural changes of epidermal growth factor that clarify the function of factor and may have potential for treatment of tumors"); and **Toby J. Mitchell,** Author of the Year (cited for "Bayesian Variable Selection in Linear Regression," a statistical study that has received various forms of recognition in the field). They are among the five Energy Systems employees who each received Martin Marietta Corporation's highest award, the silver Thomas Jefferson Cup.

ORNL researchers who received the Inventor Award, which recognizes innovative employee contributions to the activities of Energy Systems, were **Tom Ferrell** (award for team effort; the other team member was **Robert J. "Bruce" Warmack**); **Claudette McKamey,** and **Tim Scott**.

ORNL employees who were given a Technical Achievement Award, which recognizes excellence of employee contributions of a scientific or engineering nature to the activities of Energy Systems, were **Gerald Alton; Pete Angelini; Michelle Buchanan; Bill Craddick** (accepted a team award for **S. E. Burnette, D. H. Cook, G. F. Flanagan, S. S. Hurt III,** and **W. E. Thomas**); **Stan David; Nancy Dudney; Chuck Garten; Ed Hagaman; Jay Jellison; Salil K. Niyogi; Don Noid; Morris Osborne** (accepted team award for **J. L. Collins, R. A. Lorenz, J. R. Travis,** and **C. S. Webster**); **Gayle Painter; Tsung-Hung Peng; Julian Preston; Grover Robinson** (accepted team award for **Richard D. Cheverton** and **William E. Pennell**); **Glenn Suter;** and **Jonathan Woodward**.

ORNL employees who received a Publication Award, which recognizes superior employee performance in the authorship of a paper, technical article, or book that represents a significant advance in the author's professional field, were **Paul Becher** (coauthors **Chun-Hway Hsueh, P. Angelini,** and **T. N. Teigs**; **Rich Leggett** (coauthor **L. R. Williams**); **Fred Meyer** (coauthors **D. C. Griffin, C. C. Havener, M. S. Huq, R. A. Phaneuf, J. K. Swenson,** and **N. Stolterfoht**); **Toby Mitchell** (coauthor **J. J. Beauchamp**); **Dave Mullins** (coauthor **S. H. Overbury**); **Randy Nanstad** (coauthors **K. Farrell, D. N. Braski,** and **W. R. Corwin**); **Steve Pennycook** (coauthor **L. A. Boatner**); **Ker Chung Shaing; Michael Fry** (coauthors **T. J. Mitchell** and **J. B. Storer**); **Larry Waters** (coauthor is **C. E. Nix**).

ORNL employees who were given a Management Support Service Award were **Gus Testerman; Donna Griffith** (accepted award for Administrative Services team at ORNL—**Philip L. Kienlen** and **James D. Mason**); and **John Hickey**.

ORNL employees who received an Operational Performance Award were **Walt Brown** (accepted team award for **Regina Stinnett**); **Roger Carlsmith; Gloria M. Caton; Jane Eggers** (accepted team award for **Eddie M. Shirley**); **Roy Fenstermaker; Mike Kuliasha; Pete Lotts; Charlie Mullis; Al Rice; Russell Robinson; Chris Scott** (accepted team award for **R. E. Helms, T. E. Myrick, L. C. Williams, S. R. Larkins, C. Thomas, M. V. Keigan,T. H. Monk, S. D. Van Hoesen,** and **F. J. Homan**); and **Mike Wilkinson**.

Jon Soderstrom received a Community Service Award, which recognizes outstanding and noteworthy performance by Energy Systems employees engaged in voluntary, uncompensated activities that benefit the community.

Todd Anderson received the University of Tennessee Chancellor's Award for Extraordinary Professional Promise.

Claudette McKamey

Salil Niyogi

Toby Mitchell

Next Issue

Research reactors and proposed national energy policies will be examined.

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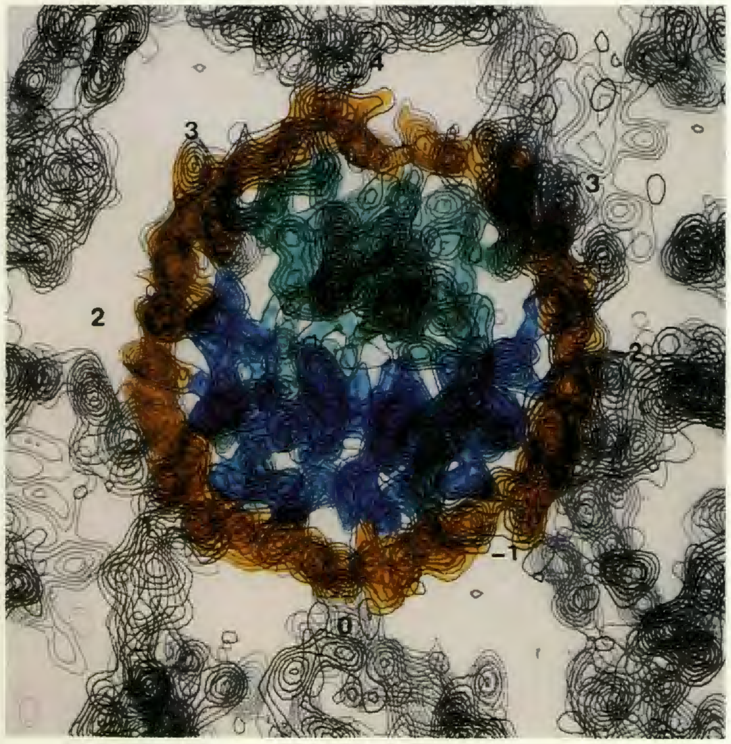
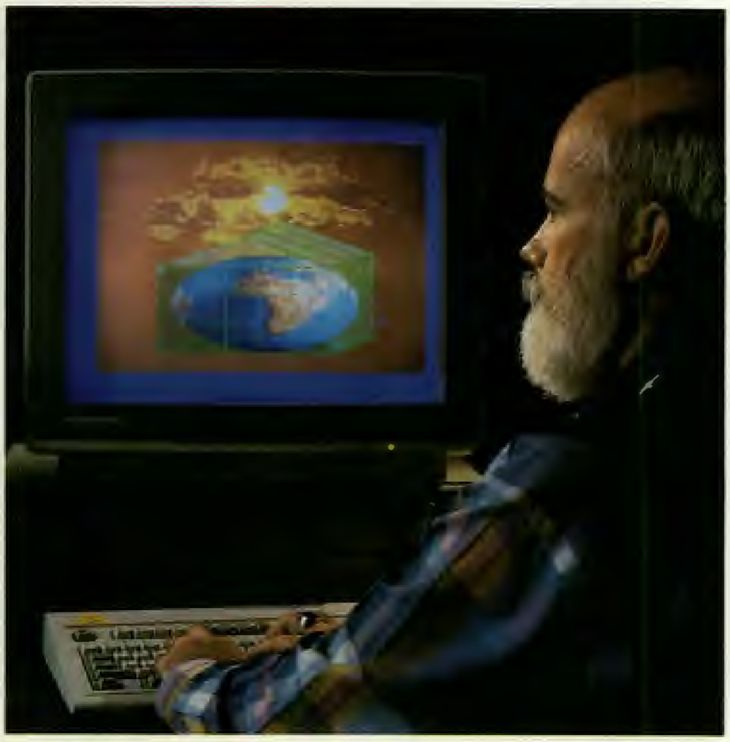


In this computer-generated representation of the structure of a chromosome unit called a nucleosome, colors define the differing electron density regions to clarify the locations and extent of core proteins and DNA. See article on "Pursuing Biology's Holy Grail" on p. 32.



Mike Farrell of the Environmental Sciences Division examines a computer depiction of the greenhouse effect. See Farrell's article on "Global Carbon Dioxide Studies" on p. 119.

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