



fiscal year 1992

annual report on
AFRRI Research

Armed Forces Radiobiology Research Institute
Bethesda, Maryland 20889-5603

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#910

On the cover

Dr. Elsa Schmauder-Chock examines bone marrow cells with an electron microscope to localize a cytokine.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 1993	3. REPORT TYPE AND DATES COVERED Annual Research Report FY 1992		
4. TITLE AND SUBTITLE Annual Report on AFRRRI Research Fiscal Year 1992			5. FUNDING NUMBERS	
6. AUTHOR(S)				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Armed Forces Radiobiology Research Institute 8901 Wisconsin Avenue Bethesda, Md. 20889-5603			8. PERFORMING ORGANIZATION REPORT NUMBER ARR-21	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Defense Nuclear Agency 6801 Telegraph Road Alexandria, Va. 22310-3398			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) This report reviews the scientific program and describes the status of in-house research at AFRRRI for the period 1 October 1991 through 30 September 1992.				
14. SUBJECT TERMS			15. NUMBER OF PAGES 176	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

SECURITY CLASSIFICATION OF THIS PAGE

CLASSIFIED BY:

DECLASSIFY ON:

SECURITY CLASSIFICATION OF THIS PAGE

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Defense Nuclear Agency
Armed Forces Radiobiology Research Institute
Bethesda, Maryland 20889-5603

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Every scientific and support department at AFRRRI contributed to this report, which covers the period from 1 October 1991 through 30 September 1992. The Information Services Department's Publications Division edited and designed the document with desktop publishing software, and its Visual Media Division prepared photographs, art, and computer graphics for the publication.

AFRRRI is accredited by the American Association for Accreditation of Laboratory Animal Care and is a member of the American Association for Laboratory Animal Science and its National Capital Area Branch and of the Scientists Center for Animal Welfare. Any protocol involving human data is approved by appropriate authorities including the Department of Defense or the Department of Veterans Affairs.

The previous report on AFRRRI research (ARR-20) covered fiscal year 1991.

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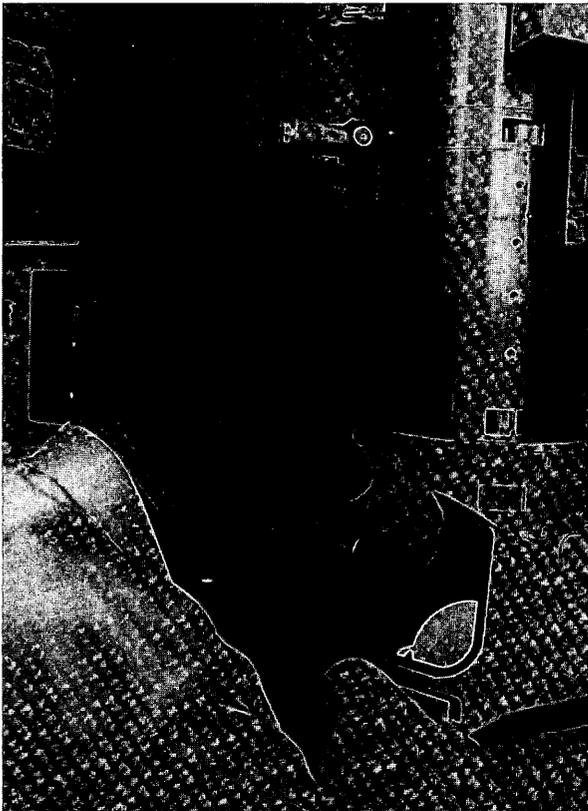
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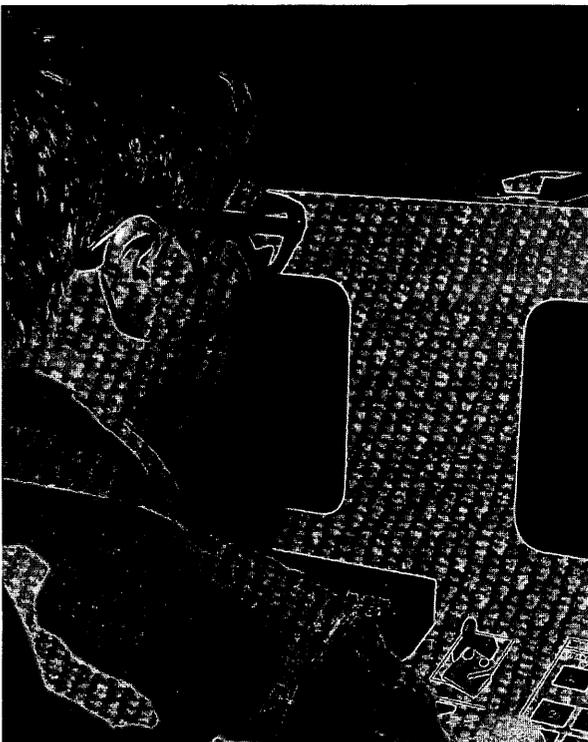


Dr. Roy Vigneulle in surgery.

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Message from the Director

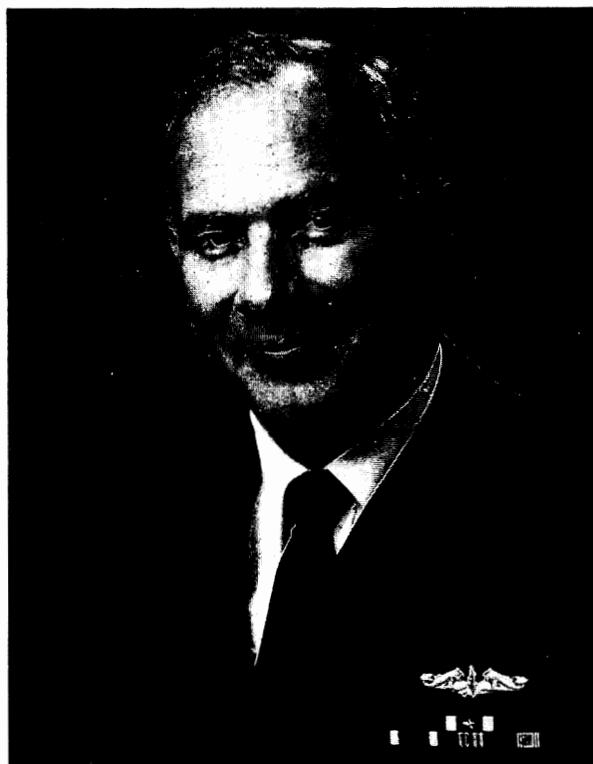
CAPT Robert L. Bumgarner, MC, USN

As AFRRRI charts its course in the evolving new world order, changes on the international scene, redirection of national assets, and changes in defense policy are providing challenging opportunities for cooperation and discovery in the field of biological effects of ionizing radiation. In the process, we are forging new alliances and pursuing innovative collaborations and techniques while maintaining our traditional role to support the defense needs of the United States and its allies.

As a triservice laboratory managed by the Defense Nuclear Agency (DNA), AFRRRI is the principal radiobiology research laboratory within the Department of Defense. AFRRRI is truly unique. There is no other array of comparable scientific capability anywhere. The Institute is equipped with a variety of radiation sources and an accredited animal facility to conduct research across the spectrum of subcellular to large-animal investigations.

Because of the changing world scene with the threat of weapons of mass destruction, which includes chemical agents and biological entities, the Institute's basic science and medical expertise is of more value to America than ever before. In this new world setting, AFRRRI arms more than 1,000 military medical professionals annually with the latest information on the effects of ionizing radiation and on casualty management. Furthermore, the Institute's various emergency medical response team members serve military and civilian needs identified by the Joint Chiefs of Staff and the National Command Authority via DNA.

With our allies, AFRRRI works through NATO, among other avenues, to advise the international community on radiation issues. We are, for example, cooperating with scientists from the newly independent states of the former Soviet Union to gather and assess human exposure data (chronic, neutron, and internal) resulting from the nuclear



events in Chelyabinsk, Russia, in programs coordinated by the Department of State. In these endeavors, the Institute is collaborating with the Department of Energy to investigate the long-term environmental and health consequences of those events.

In another arena, AFRRRI is collaborating with scientists from military facilities as well as academia and civilian laboratories to assist the National Aeronautics and Space Administration's quest for knowledge of particle carcinogenesis, so that safer manned exploration of the Moon and Mars may be undertaken. Collaborators include scientists from Columbia University, New York University Medical Center, the University of Texas Medical Branch, and the University of Wisconsin as well as from the

National Cancer Institute and the Oak Ridge and Argonne National Laboratories and from the Uniformed Services University of the Health Sciences (USUHS).

AFRRI, at the request of The Surgeon General of the Army, is developing patient monitoring and treatment protocols for Operation Desert Storm veterans who have been exposed to or injured by depleted uranium (DU). Concomitantly, the Institute is initiating research protocols to assess the long-term impact of DU fragments.

AFRRI research will continue to evolve as we move on Oct. 1, 1993, from DNA to become a

mission-oriented and a customer-based operation under the management of USUHS. The change will clearly provide the opportunity to demonstrate the broader applications of AFRRI research while exploiting the efficiencies of modeling and mechanistic studies. Radiation effects research has hardly had more interesting times than the present, which is underscored by the world political climate.

This report provides a detailed look at AFRRI research programs and our support activities; we hope it allows you to see how the Institute fits into the national research and development effort.

Foreword

Scientific Director E. John Ainsworth, Ph.D.

Scientifically, managerially, and organizationally, 1992 must be considered a defining time in the history of AFRRRI. With the assistance of the scientific department chairmen and excellent cooperation of the staff, a complete peer review of the entire scientific program was accomplished for the first time. Under the leadership of our program and project managers, comprehensive research proposals were prepared, based on the program project guidelines provided by the National Institutes of Health; project site visits occurred; presentations were made; and written critiques were provided me. Various programmatic and organizational changes are defined and are being implemented.

Dissolution of the Warsaw Pact and events in the former Soviet Union (FSU) are having a major impact on the definition of research priorities and the scope of research activities in the Department of Defense (DoD). As a command of the Defense Nuclear Agency (DNA), we are part of a DoD-wide process whereby research roles and requirements are being reassessed from the perspective of a new world order.

Together with DNA management, the senior scientific leadership at AFRRRI has critically evaluated its own program and embarked on strategic planning to define the most important scientific questions and a broad research framework that are directly relevant to the military services in the context of the new world order.

Interdepartmental working groups have been established in the following areas: impact of the new world order, neutron biophysics, performance and physiological alterations, and new initiatives that consider DoD program requirements and opportunities outside the traditional boundaries of radiobiology. Finally, discussions continue on the need for AFRRRI scientists to verify predictive models of radiation damage to biological systems in connection with underground nuclear tests. Paradigms are changing, and the AFRRRI research program will be responsive to new requirements.



In the former Soviet Union, the number of human exposures due to environmental contamination with radioisotopes and potentially toxic chemicals are dramatic. Much is to be learned from studying these populations. AFRRRI financially supported a conference between U.S. and FSU scientists at George Mason University, and I contributed to a joint U.S./FSU workshop at the University of California at Davis. Working closely with DNA and an interagency coordinating group, AFRRRI is promoting scientific contacts in the former Soviet Union with a view to establishing research contracts that will serve as a first step in archiving data already collected on exposed humans.

We also plan to support new pilot studies by FSU scientists that will help elucidate mechanisms of radiation damage and will support improved estimates of dose to irradiated persons and radiation risks for cancer, genetic damage, cataracts, and other potentially adverse health effects that occur

late in life. Research efforts on late effects of radiation in the former Soviet Union will complement ongoing mouse experiments at AFRRRI where life-span shortening after exposure to gamma rays or 600-MeV/amu iron particles is being evaluated. Studies on late effects, including carcinogenesis, will assume greater importance in the AFRRRI research program because of a dearth of a DoD-related research focus on health effects related to environmental contamination with radioisotopes, chemicals, and/or a combination of the two.

Recent DoD guidance provides that AFRRRI will become attached to the Uniformed Services University of the Health Sciences (USUHS) on Oct. 1, 1993. AFRRRI will transfer as a discrete research entity, and the AFRRRI director will report directly to the president of the university. Increased interaction with USUHS is expected to benefit AFRRRI and provide many new opportunities for research collaborations and the option of exploring new funding opportunities outside of DoD. New funding will be essential because large cuts in appropriated funds are projected over the next five years. AFRRRI and other DoD entities will transition to defense-based operational funding (DBOF) whereby the customer provides funding directly to the service provider.

Research requirements from the military services or DoD agencies will be accompanied by funding. Additionally, AFRRRI hopes to continue some aspects of the long-standing and highly productive complementary relationship with DNA. My belief is that radiation exposures, alone or together with other environmental toxicants, are implicit in the new world order where regional conflicts rather than strategic nuclear exchanges will predominate.

A great deal of praise is due our department chairmen and project managers because increased levels of responsibility and authority have been delegated to them for program execution, evaluation, and development. The major scientific accomplishments of the chairmen, managers, and their staffs are summarized in this report.

The senior management at AFRRRI and the cadre of highly motivated creative scientists constitute a research organization that exists to maintain core expertise in radiobiology and, thereby, to support the future needs of the military services. Experience, insight, and imagination will continue to be refocused on the new paradigms of the twenty-first century.

Performance Management Program

Alan H. Harris, Ph.D., Behavioral Sciences Department

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Program goals

- Determine the mechanisms for a variety of physiological dysfunctions related to performance decrements.
- Determine the behavioral changes for simple to complex performance tasks related to radiation exposure.
- Predict individual and crew performances in stressful conditions with radiation exposure.
- Evaluate radioprotective compounds for performance-degrading side effects.

Requirement

The ability of an individual to carry out the thousands of physical and cognitive tasks in the normal pursuit of daily life is often taken for granted until illness, injury, or stress degrade that ability. It's in the loss of an ability that its importance becomes obvious. The measurement of performance and its decrement after radiation insult and the prediction and/or prevention of functional loss are the principal goals of this program. The final common controlling pathway for behavioral changes resides in the central nervous system of the individual. The normal function of the nervous system is directly related to the normal function of all the other organ systems of the body, for example, the endocrine,

cardiovascular, gastrointestinal, and renal systems as well as the cellular components of the nervous system itself. Metabolism, blood pressure, blood electrolytes, and circulatory system factors all affect the function of the nervous system and, consequently, individual performance.

The military services require information about the performance-degrading effects of a potential radiation insult and about the effect of the insult on individual and crew performance. The rate of onset and the intensity of such performance-degrading symptoms as nausea, vomiting, weakness, fatigue, and diarrhea are known to some extent; but the underlying mechanisms are less well known. To develop better treatments or preventative measures, it is essential that the mechanisms be determined. In addition, proper military planning requires information about the possible performance-degrading effects of potential treatments and radioprotectors.

Strategy

An integrated, interdepartmental strategy addresses the research questions relevant to the military mission of AFRRI. The scientific approach uses behavioral, pharmacological, and physiological studies. Research animal species for these studies range from invertebrates to awake, functioning vertebrates.

Mechanisms of radiation-induced nervous system dysfunction

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Collaborator

Project 00105

This project examines the effects of ionizing radiation on the nervous system's physiological processes at the cellular, network, and integrated whole-animal levels. Our goals are to understand the cellular basis for radiation-induced deficits and, eventually, to identify protective agents that would prevent or mitigate the effects.

Although the central nervous system is considered resistant to ionizing radiation, exposure to relatively low doses is known to alter behavior. In humans, fatigue and weakness occur after exposure to doses of 1 Gy; disori-

entation is elicited by doses of 5 Gy (Anno et al., 1989).

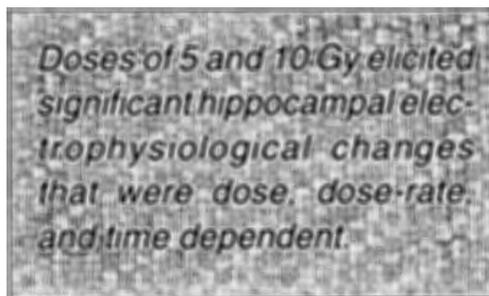
To elucidate the neuronal mechanisms responsible for acute radiation-induced deficits at low doses, we evaluated hippocampal brain slices isolated from guinea pigs at varying times after exposure to in vivo whole-body gamma (γ) radiation. Doses of 5 and 10 Gy elicited significant hippocampal electrophysiological changes that were dose, dose-rate, and time dependent. With exposure to 5 Gy, a decrease in synaptic efficacy occurred early and lasted through at least day 3; but with exposure to 10 Gy, enhanced synaptic efficacy dominated through day 1. At 1 Gy/minute, 5 Gy enhanced spike generation through day 3 with recovery to control at day 5. At 20 Gy/minute, both 5 and 10 Gy caused an early decrease, with recovery at day 1 and day 3, respectively, and then a decrease at day 5. The net output of the hippocampus was generally reduced for 30 minutes postirradiation with recovery to or beyond control at day 1. Deficits at days 3 and 5 were dose and dose-rate dependent (fig. 1).

With radiation exposure in vivo, the neural networks would be subjected to the consequences of damaged blood-brain barrier (Trnovec et al., 1990), decreased regional cerebral blood flow (Chapman and Young, 1968; Cockerham et al., 1991), and reduced systemic blood pressure (Cockerham et al., 1991; Bruner 1977). However, since we observed that the consequences of radiation are still in evidence when the tissue is removed from the animal, persistent secondary effects on the electrophysiological properties of neurons are indicated.

In contrast, in vitro, hippocampal slices are maintained in a well-controlled environment where temperature, oxygenation, and nutrients are kept at normal levels. Studies on isolated tissue reflect the effects of radiation directly on the neurons and their immediate environment. Under these conditions,

our previous studies indicated that moderate doses (>25 Gy) at dose rates of 1.5 Gy/minute and greater could directly alter the neurophysiological properties of the hippocampal slice (Tolliver and Pellmar, 1987; Pellmar et al., 1990).

We recently observed that, under these conditions,



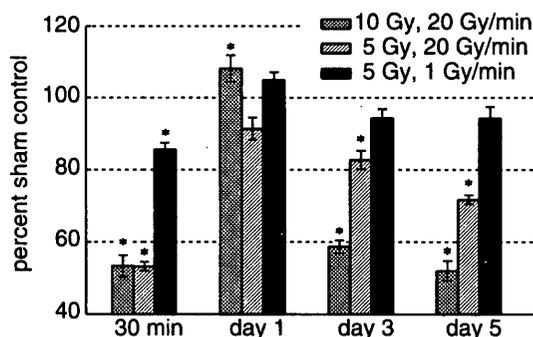


Fig. 1. Effects of whole-body γ radiation on the composite input-output of isolated hippocampal tissue 30 minutes, 1 day, 3 days, and 5 days postirradiation. All radiation effects were compared with appropriate sham controls (100%). Significant differences are indicated by asterisks ($p < 0.05$).

the effects of ionizing radiation are remarkably dose-rate dependent. At dose rates lower than about 2.5 Gy/minute, x radiation enhanced the synaptic potentials. At higher dose rates, it decreased synaptic potentials (fig. 2) as did γ radiation. On the other hand, the ability to generate spikes did not show dramatic dose-rate dependence. Multiple radiation targets with various radiation sensitivities are likely to underlie the observed patterns of damage.

Electron paramagnetic resonance spectroscopy measurements of homogenates of hippocampal slices treated with the spin trapping agent DMPO (5,5-dimethyl-1-pyrroline-1-oxide) and peroxide have shown that hydroxyl and carbon-centered free radicals are formed by interactions involving the exposed tissue and hydrogen peroxide. Peroxide generates hydroxyl radicals at specific sites (e.g., where iron reduces it). In contrast, x and γ radiation produce free radicals uniformly throughout the aqueous environment of the tissue. Since alpha (α) radiation yields 2-3 times more peroxide than x or γ radiation, studies with peroxide may be a good model for effects of high-linear-energy-transfer radiation.

We have postulated that radiation and free radicals exert some of their effects through metabolic disruption. We find that the metabolic inhibitor iodoacetate, like γ radiation and free radicals, reduces synaptic efficacy in the hippocampal brain slice. The glial specific antagonist, fluoroacetate, also is effective. The ability to generate spikes is minimally altered by the metabolic blocker. These data support our hypothesis and further suggest a role for glial cells in the free-radical-induced neurophysiological deficits.

We found previously that free radicals decrease synaptic transmission, in part, through a decrease in presynaptic release of a neurotransmitter. In addition to decreasing the calcium-dependent evoked release, free radicals enhance the calcium-independent basal release (Gilman et al., 1992).

To evaluate this second action, we used [^3H]-D-aspartate, which is taken into the cytoplasm but not into the synaptic vesicles. We found that non-vesicular release of the amino acid was enhanced by hydrogen peroxide. The iron chelator desferal blocked this effect, suggesting that peroxide acts solely through its interaction with iron to form hydroxyl free radicals. Addition of iron was not necessary to elicit the peroxide actions; there is sufficient iron in the tissue to generate the radicals. Chloramine-T, a protein-oxidizing agent, was able to mimic the enhancement of nonvesicular release of a neurotransmitter. As with the decrease in synaptic transmission (Pellmar and Neel, 1989), the increase in basal release is a consequence of a protein oxidation reaction rather than of lipid peroxidation.

We also are exploring the role of the hypothalamic-pituitary-adrenal (HPA) axis in the whole-animal response to ionizing radiation. The HPA is the primary neuroendocrine system that responds to physical and psychological stressors and is believed to be crucial to survival during severe challenges. We found that brain catecholamines specifically stimulate the subset of hypothalamic neurosecretory cells (Whitnall 1990) that respond to stress but have no effect on the neurons that respond selective-

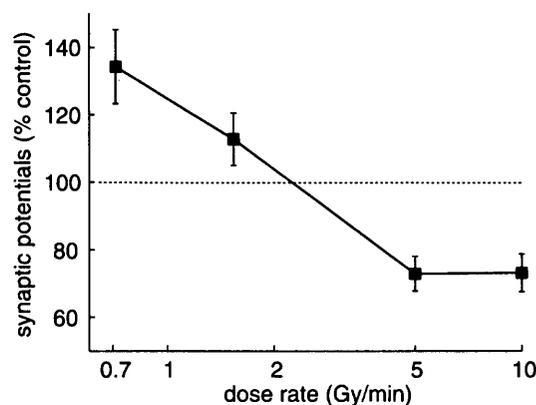


Fig. 2. Dose rate sensitivity of synaptic potentials in hippocampal brain slice. Tissue was exposed to 50 Gy x radiation at a variety of dose rates ranging from 0.7 to 10 Gy/minute. Data were collected approximately 65 minutes after the beginning of exposure. The lower dose rates enhanced the synaptic potential while the higher dose rates inhibited the synaptic potential.

ly to inflammatory stimuli. In Lewis rats, which possess defective HPA responses to inflammatory stimuli, catecholamine inputs were normal, but hypothalamic expression of the peptide vasopressin was deficient.

Within hours after exposing rats to 8.5 Gy γ radiation, we observed a pronounced, but short-lived, elevation of circulating glucocorticoids, compared with that in sham-irradiated rats. Seven days after the exposure and before any behavioral signs of radiation sickness were observed, the rats exhibited abnormal plasma glucose and ACTH (adrenocorticotrophic hormone) in response to endotoxin and insulin (fig. 3). No changes in baseline glucose or ACTH levels were apparent at that time. When behavioral effects were evident, starting about 12 days postirradiation, plasma glucocorticoids were not elevated. Three sex-steroid processing enzymes were found in the glucocorticoid-synthesizing layers of the adrenal cortex, which may be related to male-female differences in glucocorticoid responses and recovery postirradiation.

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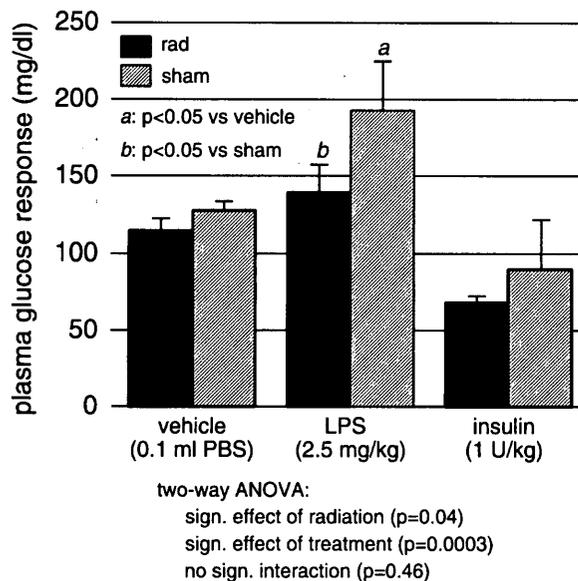


Fig. 3. Plasma glucose responses to bacterial lipopolysaccharide (LPS) endotoxin or insulin in rats 7 days after 8.5-Gy whole-body γ radiation. Vehicle is phosphate-buffered saline (PBS). Values are mean \pm SEM (standard error of the mean) of 3 rats per group. Probability levels represented by "a" and "b" were obtained by one-factor ANOVA (analysis of variance).

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Radiation effects on complex task performance

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Project 00178

The primary goals of this project are (1) to identify specific behaviors and behavioral processes that are most susceptible to disruption by ionizing radiation, (2) to determine how behavioral and environmental variables modify radiation-

induced performance decrements, and (3) to evaluate radiobiological and pharmacological factors that may mediate or modulate effects of radiation on performance.

To achieve our goals, we emphasize the measurement of radiation-induced changes in schedule-controlled operant behavior (i.e., rule-governed behavior that is established and maintained by the delivery of response-contingent positive or negative reinforcers). Two lines of operant behavioral research are the effects of ionizing radiation on learning and the modification of radiation effects by environmental stimuli.

The effects of ionizing radiation on learning remain poorly defined despite the fact that radiation-induced disruptions in performance have been studied for many years (Kimeldorf and Hunt, 1965). We have assessed the effects of gamma (γ) radiation on learning in rats performing a repeated acquisition task. The task requires a subject to press three response keys in the correct sequence to obtain a food pellet. The correct sequence changes daily. Learning, defined as daily acquisition of the correct response sequence, is indicated by a decrease in a subject's rate of errors during a given session.

Gamma radiation produced a dose-dependent disruption of learning in rats as assessed by changes in repeated acquisition performance (fig. 1). Doses of 4.5 and 8 Gy (bilateral exposures at a dose rate of 2.5 Gy/minute) decreased response rates and increased errors, but doses of 1 and 3 Gy were

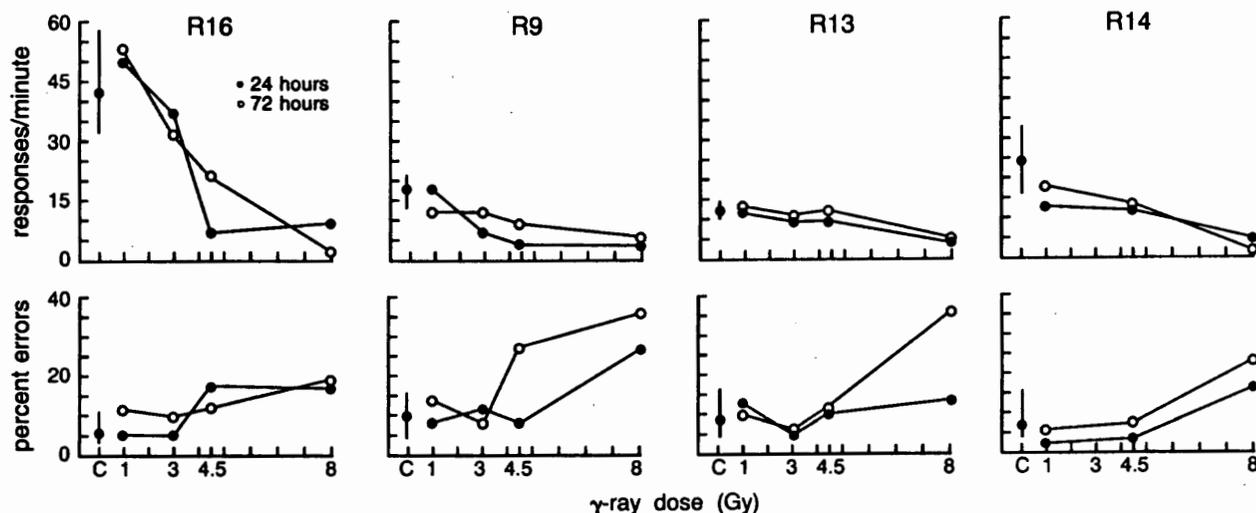


Fig. 1. Effects of ^{60}Co γ rays on the response rates and percent errors for each of four rats (R16, R9, R13, R14) responding under a repeated acquisition procedure. The filled point and vertical line above C indicate the control data mean and range respectively.

generally without effect (Winsauer and Mele, in press). It is noteworthy that error rates at a given dose were differentially affected as a function of time after irradiation; the number of errors was greater at 72 hours than at 24 hours. In contrast, response rates were decreased relatively consistently or were decreased less at 72 hours than at 24 hours. These results indicate that the repeated acquisition procedure allows for a separation of radiation effects on learning (i.e., errors) from more general effects on performance (i.e., response rates). This study also demonstrates the importance of the use of single-subject experimental designs to evaluate the effects of ionizing radiation on learning.

Behavioral disruptions induced by certain drugs and toxicants can be attenuated by strong environmental stimulus control; that is, by an environment that signals the organism when and where to respond. Previously, in the annual report on AFRRRI research for fiscal year 1991, we reported that effects on performance in rats, following acute exposure to γ radiation (4.5-7.5 Gy), were attenuated by strong stimulus control. Recently, we have begun to examine behavioral effects of multiple, low-dose exposures under different degrees of stimulus control.

In our laboratory, fixed consecutive number (FCN) schedules of reinforcement are used to establish differential degrees of stimulus control. The FCN schedule requires subjects to press two levers a predetermined number of times in a specific sequence. Under one FCN schedule, highly discriminable cues (lights and a tone) are presented to indicate when and where to respond. Under a second FCN schedule, these cues are absent and appropriate responses are controlled by internal rather than external (environmental) stimuli.

To initiate our evaluation of effects of repeated low-dose exposures on complex performance, rats responding under these two FCN schedules were irradiated six times at 1-week intervals with 3 Gy of ^{60}Co γ rays (bilateral exposures at a dose rate of 2.5 Gy/minute). In general, disruptions of performance (i.e., decreased response rates and increased error rates) became more severe with successive expo-

sure, indicating that cumulative disruptions in complex performance can occur after repeated low-dose exposures. We also observed that the size of the disruptions were frequently smaller under the cued FCN schedule than under the noncued schedule. These findings indicate that strong environmental stimulus control can attenuate the disruptive effects of γ radiation on complex performance. Animals currently in training will be used to evaluate lower radiation doses (e.g., 1.5 Gy) given repeatedly over extended time periods (e.g., 12 weeks).

[Our] findings indicate that strong environmental stimulus control can attenuate the disruptive effects of γ radiation on complex performance.

To evaluate effects of ionizing radiation on complex performance in a higher species, rhesus monkeys have been trained on variants of the FCN schedule and the repeated acquisition procedures. In initial studies with monkeys, we have focused on establishing the sensitivity of

the baselines generated by these procedures by administering several prototypical compounds having short-term, reversible effects. For example, pentobarbital produced selective disruptions in performance maintained under cued and noncued FCN schedules of food reinforcement. More specifically, pentobarbital increased the error frequency under the noncued FCN schedule at doses that did not alter error frequency under the cued FCN schedule.

In another primate study involving a repeated acquisition task, a schedule with both learning and performance components served as a baseline. In the learning component, subjects were required to learn a new sequence of behavior each day (see section above describing repeated acquisition in rat studies). In the performance component, subjects were required to perform the same sequence of behavior each day. Buspirone, a drug with antiemetic, appetite-stimulant, and anxiolytic properties, produced dose-dependent disruptions in both components as shown by decreased response rates and increased errors. However, response in the learning component was disrupted to a greater degree and at lower doses than response in the performance component. These findings indicate that both the FCN and repeated acquisition procedures generate behavioral baselines that are sensitive to, and selectively disrupted by, certain drugs. Additional monkeys are being trained for use in the evaluation of effects of sublethal doses of γ radiation.

The identification and characterization of pharmacological agents that may be useful in the treatment of radiation exposure is an ongoing concern. We have conducted several studies on 5-HT₃ antagonists, a relatively new class of drugs that are highly effective against cytotoxic drug- and radiation-induced emesis. Our studies addressed several aspects of the behavioral effects of 5-HT₃ antagonists. Studies completed to date include those that used food intake, conditioned taste aversion, and schedule-controlled operant performance as primary dependent variables to characterize 5-HT₃ antagonists.

Loss of appetite and decreased food intake are two of the most sensitive indices of exposure to ionizing radiation in humans and animals. Because several 5-HT₃ antagonists (e.g., ondansetron, zacopride, eusatron) have been shown to be highly effective against radiation-induced emesis (King and Landauer, 1990; Rabin and King, 1992), we tested the efficacy of these compounds against a radiation-induced eating deficit that is likely to be mediated, at least in part, by effects on the gastrointestinal system. For example, ondansetron was administered to rats after a single exposure to 4.5 Gy of ⁶⁰Co γ radiation, a dose that reliably decreases food intake postexposure. However, none of the doses (0.1-1 mg/kg) of ondansetron tested were effective in reversing the suppression of eating at any time (4-96 hours) postexposure. Concomitantly, ondansetron did not alter food intake in non-irradiated rats. In contrast, 8-OH-DPAT (0.1-1 mg/kg), a relatively selective 5-HT_{1A} receptor agonist, increased food intake in both irradiated and sham-irradiated rats (fig. 2).

These findings suggest it is unlikely that radiation-induced anorexia in rats is the result of 5-HT₃-receptor-mediated effects. In addition, these findings indicate that radiation does not appear to interfere with eating mechanisms that are sensitive to stimulation by 8-OH-DPAT. The results with 8-OH-DPAT, together with similar results for the benzodiazepine chlordiazepoxide (as reported in the annual report on AFRRRI research for fiscal year 1991), suggest potential pharmacological treatments for radiation-induced anorexia.

A second study used conditioned taste aversions (CTAs) in rats to evaluate effects of 5-HT₃ antagonists. The CTA paradigm pairs the ingestion of a normally preferred substance (e.g., a saccharin

solution) with the administration of a drug or toxicant. A CTA is indicated when animals avoid ingestion of the substance when it is presented subsequently in the absence of the drug or toxicant. The CTA paradigm provides an objective and quantifiable means for evaluating interactions between a wide variety of treatments including those shown to have emetic or antiemetic properties (e.g., Grant, 1987).

In our study (Mele et al., 1992), CTAs in rats were established with low doses of the chemotherapeutic drug cisplatin, which is highly emetic in mammals that vomit. Neither ondansetron nor zacopride blocked or attenuated the formation of the cisplatin-induced CTA under a variety of dosing conditions. Similarly, both ondansetron and zacopride were ineffective against a CTA induced by a low dose of lithium chloride, a drug that is emetic in ferrets (Rabin and Hunt, 1992). Thus, rats, in which CTAs are induced by agents that are emetic in other species, are not sensitive to treatment with antiemetic drugs acting at 5-HT₃ receptors. These findings suggest that activation of 5-HT₃ receptors does not underlie the induction of CTAs by either cisplatin or lithium chloride.

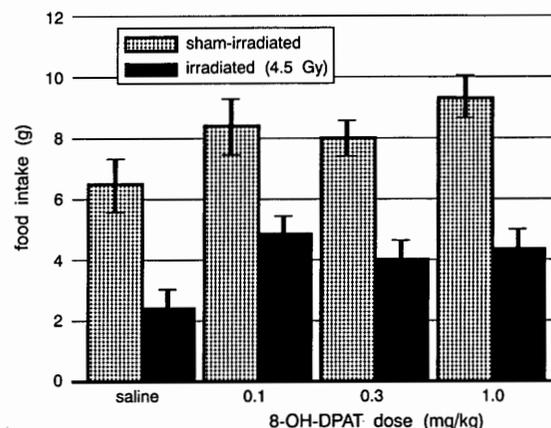


Fig. 2. Effects of 8-OH-DPAT on mean food intake in irradiated and sham-irradiated groups of rats (N=8/group). Data are the average of five consecutive daily 60-minute test sessions. Group averages are the mean of individual averages for the five days; vertical lines indicate ± 1 SEM (standard error of the mean). The test session on day 1 began 4 hours after either sham irradiation or irradiation with 4.5 Gy of γ photons. Either 8-OH-DPAT or saline were injected intraperitoneally 20 minutes before each test session.

In contrast to the results obtained with the 5-HT₃ antagonists, the glucocorticoid dexamethasone was highly effective in blocking the CTA induced by a low dose of cisplatin (fig. 3). Dexamethasone has also been shown to attenuate a radiation-induced CTA in rats (Cairnie and Leach, 1982) and to reduce chemotherapy-induced emesis (Al-Idrissi et al., 1988). Taken together, these results indicate that CTAs in rodents may be useful in the investigation of certain types of antiemetic treatments.

In a third study, we evaluated the behavioral toxicity of 5-HT₃ antagonists by determining the effects of these drugs on schedule-controlled operant behavior. As stated in the annual report on AFRRRI research for fiscal year 1991, the 5-HT₃ antagonists ondansetron, zacopride, and ICS 205-930 did not alter the performance of rats responding under a multiple fixed interval, fixed ratio (MULT FI FR) schedule of milk reinforcement except at high doses (>10 mg/kg). Recently, we have begun investigating effects of ICS 205-930 administered in combination with a number of other drugs. Our intent is to determine if 5-HT₃ antagonists interact in a detrimental manner with drugs used for therapeutic or performance-enhancing purposes. So far, we have evaluated the effects of haloperidol (an

antipsychotic and antiemetic adjunct), caffeine (a psychomotor stimulant), and chlordiazepoxide (an anxiolytic, anticonvulsant, antiemetic adjunct, and appetite stimulant), each given in combination with ICS 205-930.

The interactions between ICS 205-930 and haloperidol are presented in figure 4. Haloperidol (0.01-0.32 mg/kg) dose-dependently decreased both FI and FR response rates. ICS 205-930 (0.1-10 mg/kg) alone did not alter response rates consistently. When ICS 205-930 was administered 45 minutes before haloperidol, both FI and FR response rates were reduced more than they were after haloperidol alone. Moreover, response rates were decreased to a greater degree as the dose of ICS 205-930 given in combination with haloperidol increased. We also evaluated the effects of these drugs and drug combinations on the FI index of curvature. The index of curvature, a measure of differential responding over time that characterizes performance under FI schedules, indicates the degree to which responding is under the control of the FI contingency. In contrast to the observed effects of haloperidol and ICS 205-930 on FI response rates, neither drug alone or in combination altered the FI index of curvature. Thus, neither haloperidol nor ICS 205-930, alone or in combination, specifically disrupted control by the FI schedule even though response rates were decreased.

When ICS 205-930 was administered prior to either chlordiazepoxide or caffeine, response rates were reduced below those obtained with chlordiazepoxide or caffeine alone. These interactions, however, were much less marked than those obtained with haloperidol. For the most part, significant interactions between ICS 205-930 and chlordiazepoxide or caffeine occurred only at the highest doses of each drug. Moreover, interactions were as likely to occur with the lowest dose of ICS 205-930 (0.1 mg/kg) as with the highest dose (10 mg/kg). Overall, these results indicate that at least one 5-HT₃ antagonist, ICS 205-930, can exacerbate the performance-disrupting effects of several types of drugs, and that these adverse interactions occur more consistently with haloperidol than with either chlordiazepoxide or caffeine.

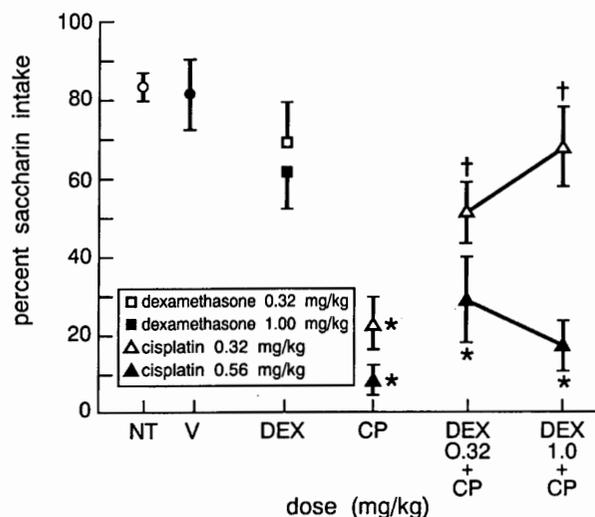


Fig. 3. Effects of dexamethasone (DEX) pretreatment on the cisplatin-induced conditioned taste aversion. Each point indicates the mean intake of 8-10 rats; error bars indicate ± 1 SEM; * indicates $p < 0.05$ versus vehicle (V); † indicates $p < 0.05$ versus cisplatin (CP). NT indicates data from a group of rats that received no treatment.

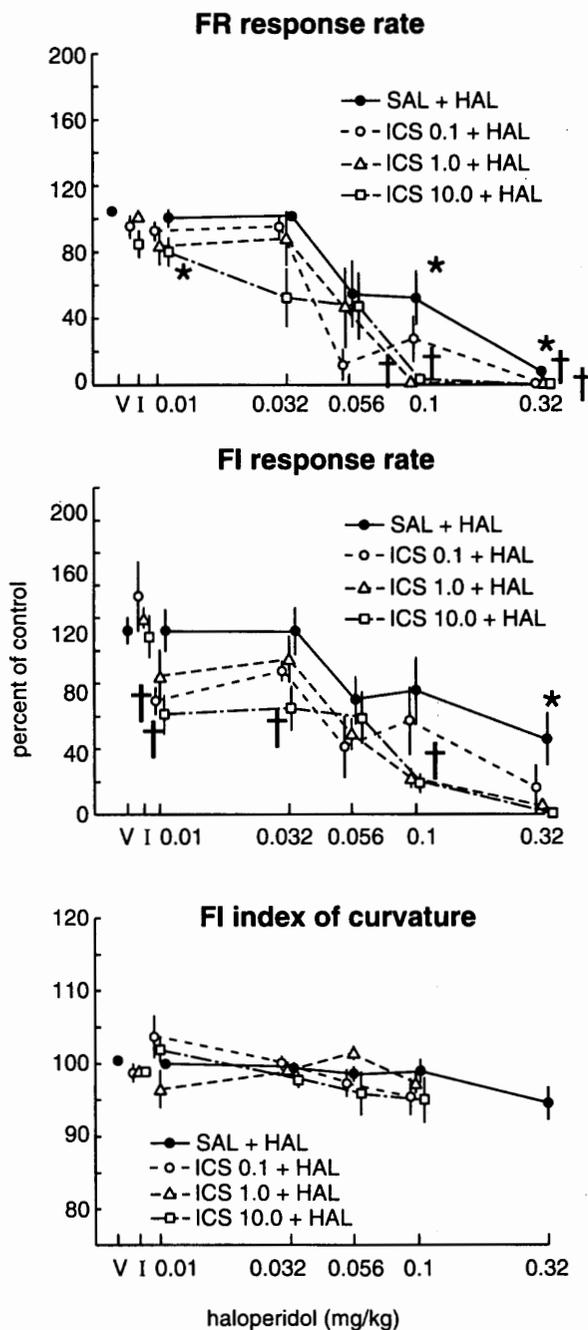


Fig. 4. Effects of haloperidol administered alone and after pretreatment with ICS 205-930 on MULT FI FR schedule performance in rats. Points above V indicate effects of saline (SAL) vehicle injections; points above I indicate effects of individual doses of ICS 205-930. Two injections were given on treatment days. Either ICS 205-930 or saline was injected 75 minutes pre-session, and either haloperidol or saline was injected 30 minutes pre-session. Data points indicate the mean (± 1 SEM) of six rats. An * indicates $p < 0.05$ versus vehicle; † indicates $p < 0.05$ versus haloperidol.

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Mele PC, McDonough JH. Evaluation of 5-HT₃ antagonists on schedule-controlled performance in rats. 21st Annual Meeting of the Society for Neuroscience, New Orleans, La., November 1991

Mele PC, McDonough JH. Stimulus control modulates effects of ionizing radiation on operant performance. 20th Annual Meeting of the Society for Neuroscience, St. Louis, Mo., November 1990

Biological mechanisms of radiation-induced performance decrements

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Project 00157

The goals of this project are to determine the mechanisms by which radiation exposure affects performance and to evaluate behavioral and pharmacological interventions that attenuate the performance-degrading effects of radiation exposure.

Hippocampus, memory, and norepinephrine

The hippocampus is a brain area important for learning, memory, and motor performance, functions that are impaired after exposure to ionizing

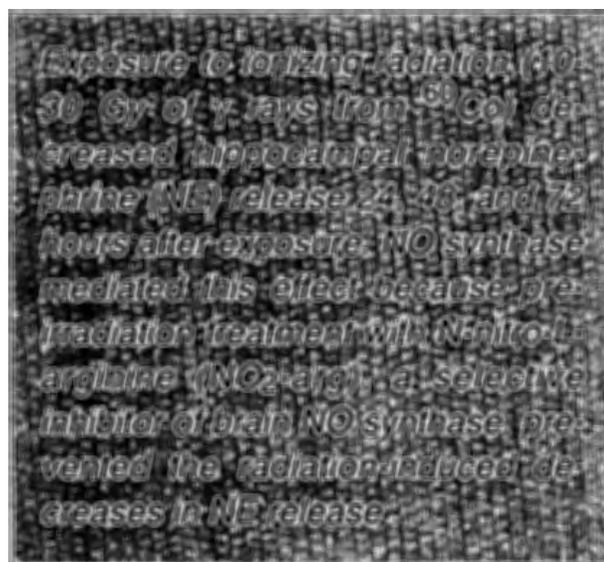
radiation. To test spatial and short-term memory associated with the hippocampus, we used the Morris water maze and an automated water-escape Y maze, a new design developed at AFRRI.

We found that, 24 hours after exposure to 15 Gy of ^{60}Co gamma (γ) radiation, the rats were slowed by a significant 40% in their remembering the location of a submerged platform in the Morris maze. This dose had no effect on the rats' swimming speed or on the rats' latency of travel to the pool quadrant with the platform.

At 36 hours after exposure to 1 Gy of 600 MeV ^{56}Fe particles, rats took twice as long to initially discover the hidden platform, a significantly longer time; however, they were not slowed in their remembering its location or in discovering and remembering new locations at 60 and 84 hours after exposure.

An hour after exposure to 7.5 Gy of ^{60}Co γ radiation, rats tested in the Y maze chose the side that had offered the escape platform on the preceding trial at the expense of choosing the correct side cued with a visual stimulus. These results support earlier reports (Kimeldorf and Hunt, 1965) that altered focus of attention may be important in explaining postirradiation changes in performance of learning and memory tasks.

Noradrenergic systems are important in mediating arousal, food intake, and to some extent motor function. Histofluorescence and immunohistochemical techniques have shown noradrenergic pathways



in the hippocampus. Ionizing radiation generates free radicals, including oxygen-derived radicals that have been implicated in cell damage following ischemia. Brain ischemia induces the release of an excessive amount of glutamate in the hippocampus, and glutamate acts on nitric oxide (NO) synthase to form NO through N-methyl-D-aspartate receptors to cause toxic effects.

Exposure to ionizing radiation (10-30 Gy of γ rays from ^{60}Co) decreased hippocampal norepinephrine (NE) release 24, 48, and 72 hours after exposure. NO synthase mediated this effect because preirradiation treatment with N-nitro-L-arginine ($\text{NO}_2\text{-arg}$), a selective inhibitor of brain NO synthase, prevented the radiation-induced decreases in NE release (Kandasamy et al., 1992). See figure 1. Treatment with $\text{NO}_2\text{-arg}$ also enhanced NE release in sham-irradiated rats, suggesting that NO is involved in the regulation of NE release under normal conditions.

Calcium and protein kinase C

An increase in intracellular calcium is critically important in the release of neurotransmitters. One calcium-dependent enzyme that is activated upon depolarization of nerve tissue is protein kinase C (PKC), and there is evidence that this activation leads to the phosphorylation of several specific proteins in nerve endings. It was shown that tumor-promoting phorbol esters, which are known to stimulate PKC, are able to stimulate the release of several transmitters in many brain areas including the hippocampus.

Gamma radiation has been shown to reduce potassium-chloride-stimulated voltage-dependent calcium uptake in whole-brain, hippocampal, and striatal synaptosomes; and studies with phorbol esters have suggested that ionizing radiation might affect the activity of PKC (Kandasamy and Harris, 1992). Of the four immunoreactive PKC isozymes ($\text{PKC}\alpha$, $\text{PKC}\beta$, $\text{PKC}\gamma$, and $\text{PKC}\epsilon$), only the $\text{PKC}\alpha$ and $\text{PKC}\gamma$ immunoreactivity are found to be significantly decreased following irradiation.

Microdialysis

Our *in vivo* microdialysis studies comparing the release of striatal dopamine and its metabolites in response to potassium-chloride depolarization

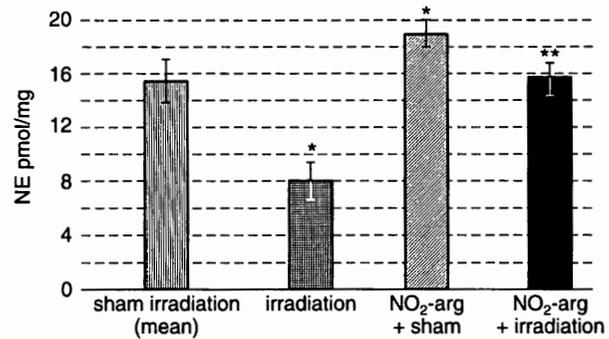


Fig. 1. Effect of treatment with 5 mg/kg $\text{NO}_2\text{-arg}$ intraperitoneally 1 hour before 10-Gy irradiation. An asterisk indicates a significant difference from sham irradiation values ($p < 0.05$). Two asterisks indicate a significant difference from irradiated values ($p < 0.05$).

between free-moving and chloral-hydrate-anesthetized rats, demonstrated that chloral hydrate anesthesia could have significant effects on the pharmacological response of the striatal dopaminergic system. The findings were presented in April 1992 at the 76th Annual Meeting of the Federation of American Societies for Experimental Biology (FASEB) in Anaheim, Calif., as "Effects of chloral hydrate on K^+ -induced striatal dopamine (DA) release in male rats."

Interleukin and radiation-induced hyperthermia

Treatment with recombinant human interleukin- 1α (rhIL- 1α) 20 hours before irradiation attenuated radiation-induced hyperthermia in rats. See figure 2. Our findings were presented in April 1992

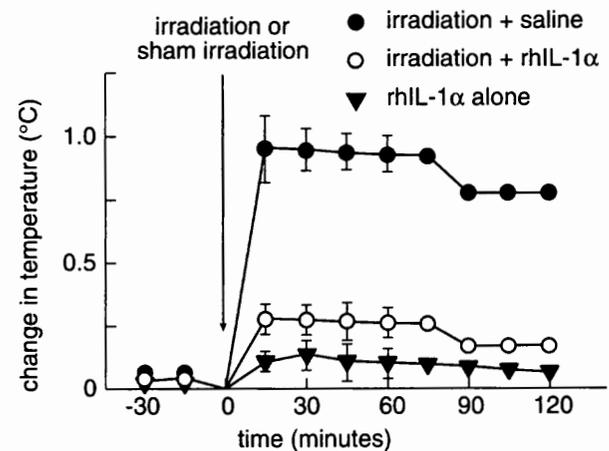


Fig. 2. Effect of treatment with rhIL- 1α or saline 20 hours before sham or 10-Gy irradiation.

at the 76th Annual Meeting of FASEB in Anaheim, Calif., as "Mechanisms involved in attenuation of radiation-induced hyperthermia in rats by interleukin."

Experiments were done to elucidate the mechanisms involved and the role of the hypophysis and adrenals in rhIL-1 α -induced attenuation of radiation-induced hyperthermia. Treatment with rhIL-1 α 20 hours before irradiation did not attenuate radiation-induced hyperthermia in adrenalectomized and hypophysectomized rats. RhIL-1 α administered 20 hours before sham or radiation exposure increased hypothalamic prostaglandin E₂ (PGE₂), superoxide dismutase (SOD), and glutathione peroxidase (GSHPx) levels. Central administration of PGE₂ 30 minutes before irradiation did not attenuate radiation-induced hyperthermia, but central administration of SOD or GSHPx did.

IL-1 α increases the levels of corticotropin-releasing hormone (CRH) and IL-6. Treatment with central administration of IL-6 30 minutes before irradiation did not inhibit radiation-induced hyperthermia, but similar treatment with CRH did. These results suggest that attenuation of radiation-induced hyperthermia by IL-1 α requires intact hypophysis and adrenals and is mediated by an increase in CRH and antioxidant enzyme levels (Kandasamy et al., 1992).

Heavy charged particles

Rats were exposed to iron (600 MeV/amu), helium (165 MeV/amu), neon (522 MeV/amu), or argon (670 MeV/amu) particles to evaluate the behavioral toxicity of these types of radiations. Behavioral toxicity was assessed by the conditioned taste aversion paradigm. Exposure to each type of radiation produced dose-dependent increases in the intensity of the acquired taste aversion. However, linear energy transfer (LET) was not a good predictor of the relative biological effectiveness of the different types of radiation, measured as the dose that produced a 50% decrease in the intake of the sucrose-conditioned stimulus (Rabin et al., 1991). We completed our study on the relationship of LET to behavioral toxicity with exposure to niobium particles (400 MeV/amu). See figure 3.

The behavioral toxicity of niobium particles is similar to that of fission spectrum neutrons and lower than that of iron particles. These results are

consistent with the hypothesis that an increase in radiation LET beyond ~200 KeV/ μ m may cause a decrease in relative behavioral effectiveness.

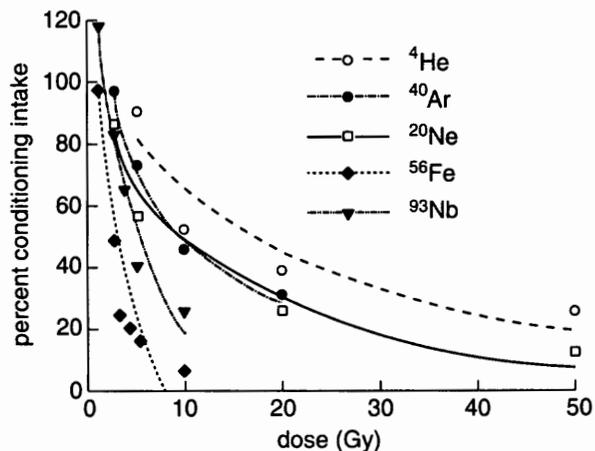


Fig. 3. Dose-response curves for conditioned taste aversion for helium (⁴He), argon (⁴⁰Ar), neon (²⁰Ne), iron (⁵⁶Fe), and niobium (⁹³Nb) radiation in rats in a series of experiments.

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Effects of radiation and radioprotectors on motor skills

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Project 00159

This project, using a variety of motor function tests, assesses the effects of ionizing radiation and radioprotectors on the ability to perform motor tasks. Our goal is to help develop radioprotective compounds that have minimal behavioral toxicity and will enable defense personnel to carry out their duties in hostile environments that include nuclear battlefields, radiation accident scenes, and manned space missions.

Studies of radioprotectors (i.e., chemical compounds that protect mammals from the lethal effects of radiation) have been conducted for more than 40 years (see reviews by Monig et al, 1990, and Weiss et al., 1990). Most, however, have focused on identification of the maximal dose reduction factor (DRF) without regard to the behavioral side effects. Our studies have used the spontaneous locomotor activity test to assess alterations in locomotion, the accelerating rotating rod test to assess balance and coordination, and the treadmill test to assess fatigability.

The spontaneous locomotor activity test has been used extensively in the fields of behavioral

pharmacology and behavioral toxicology (MacPahil et al., 1989). Success with it has led a number of agencies, including the Environmental Protection Agency and the World Health Organization, to recommend it as a behavioral assay to evaluate chemical agents (Sette and Levine, 1986; Tilson and Moser, 1992).

Using the activity test with rodents, we showed that locomotor decrement is induced by ionizing radiation (Landauer et al., 1988) and by many radioprotectors (Landauer et al., 1990, 1991, 1992).

Furthermore, in related experiments, we found that the methylxanthine caffeine mitigates radiation-induced locomotor decrement. In an initial experiment, we examined the effects of acute, whole-body, bilaterally administered gamma (γ) radiation (^{60}Co) on locomotor activity in male CD2F1 mice on the day of irradiation. The mice were tested in an automated activity monitor (Omnitech Electronics, Columbus, Ohio) during the dark period of the light/dark cycle. A 7-Gy sublethal dose of γ radiation delivered at a dose rate of 1 Gy/minute resulted in significantly decreased locomotor activity. Intraperitoneal (i.p.) administration of anhydrous caffeine (1,3,7-trimethylxanthine) in doses of 5, 10, 20, or 40 mg/kg immediately before irradiation prevented the radiation-induced locomotor decrement for 1, 2, 3, and 4 hours, respectively. The effect of 40 mg/kg caffeine on radiation-induced locomotor decrement is illustrated in figure 1.

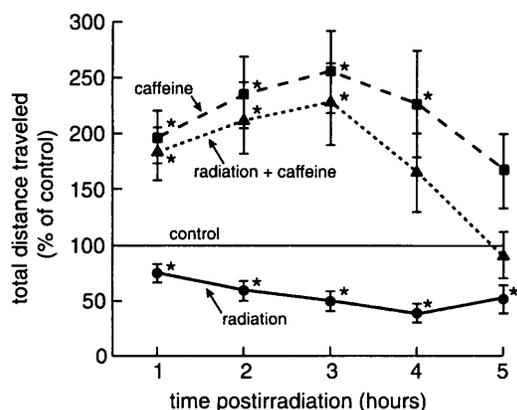


Fig. 1. Effect of 40 mg/kg (i.p.) of caffeine on radiation-induced locomotor decrement in mice tested on the day of irradiation. Mice received a single injection of caffeine immediately before receiving a 7-Gy dose of ^{60}Co radiation. The solid line at 100% represents the saline vehicle control group. The vertical bars represent the SEM (standard error of the mean). Asterisks indicate $p < 0.05$ from the vehicle control group.

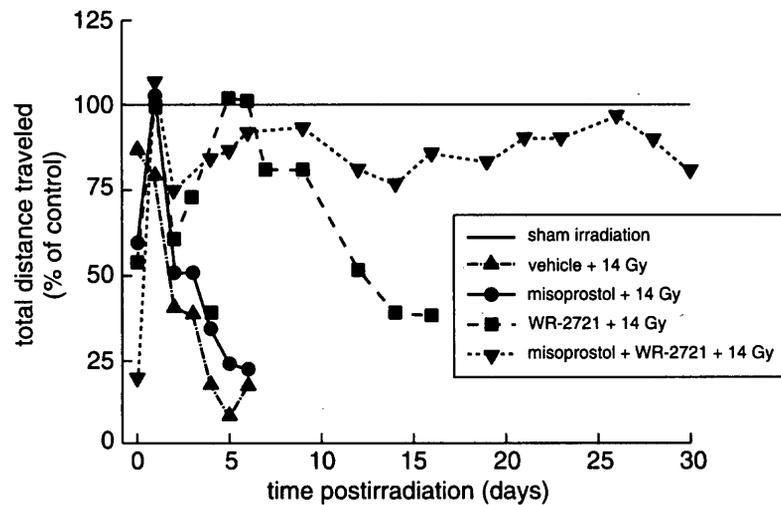


Fig. 2. The effect of the prostaglandin analogue misoprostol alone or in combination with WR-2721 on the locomotor activity of irradiated mice (14 Gy ^{60}Co). In this study none of the mice treated with misoprostol alone or WR-2721 alone survived; all of the mice treated with the combination of misoprostol + WR-2721 survived.

In a subsequent experiment, repeated administration of caffeine (20 mg/kg, i.p.) every 48-72 hours for 14 days to mice irradiated with 7 Gy ^{60}Co did not result in tolerance to the stimulatory effects of caffeine. Thus, caffeine continued to mitigate radiation-induced locomotor decrements on the days of administration.

We also studied the radioprotective and behavioral effects of the prostaglandin analogue misoprostol, which has been shown to protect intestinal clonogenic cells when administered alone and in combination with WR-2721 (Hanson et al., 1988). A series of experiments, supported by a research grant from the Department of Veterans Affairs, evaluated the effects of misoprostol alone and in combination with WR-2721 in CD2F1 male mice. The $\text{LD}_{50/30}$ (95% confidence limits) for vehicle was 7.83 (7.67-7.98) Gy. For misoprostol alone (1 mg/kg, subcutaneously, 2 hours before ^{60}Co irradiation at 1 Gy/minute) the $\text{LD}_{50/30}$ was 7.95 (7.32-8.16) Gy, yielding a DRF of 1.02. When WR-2721 (200 mg/kg, i.p., 30 minutes before irradiation) was administered alone and in combination with misoprostol, the $\text{LD}_{50/30}$'s were 13.79 (13.56-14.05) Gy and 15.95 (15.66-16.28) Gy, respectively, yielding a DRF of 1.76 for WR-2721 and 2.04 for misoprostol + WR-2721 (fig. 2).

Locomotor activity was evaluated in mice pretreated with misoprostol, WR-2721, or the combination and then exposed to a 14-Gy dose of ^{60}Co γ radiation. Locomotor behavior was recorded for 1

hour on each test day throughout the 30-day experiment. All groups of mice exhibited decreased motor behavior during the first hour postirradiation (day 0), recovery 24 hours later, and then a second decline beginning 48 hours postirradiation (fig. 3). The locomotor decrement on the day of irradiation was significantly greater in misoprostol + WR-2721-treated mice compared with WR-2721-treated mice. After the second decline, mice treated with misoprostol + WR-2721 recovered at 96 hours and continued to exhibit locomotor behavior com-

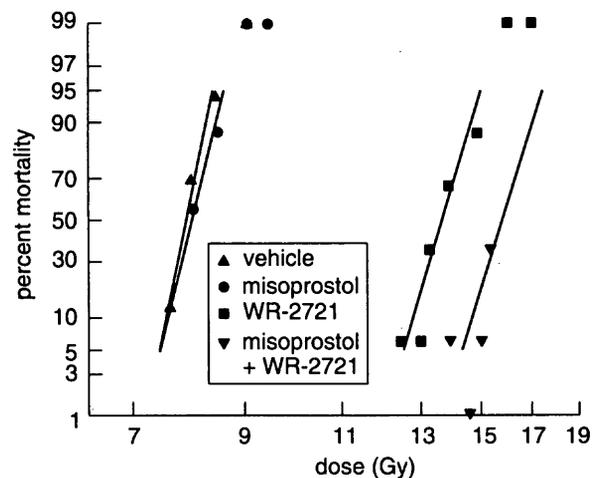


Fig. 3. Effect of ^{60}Co irradiation on 30-day survival in mice treated with vehicle, misoprostol, WR-2721, or misoprostol + WR-2721. Misoprostol (1 mg/kg, subcutaneously) was administered 2 hours before irradiation, WR-2721 (200 mg/kg, i.p.) was given 30 minutes before irradiation.

parable to that of sham irradiated control mice. All other irradiated groups (misoprostol alone, WR-2721 alone, vehicle alone) continued to show a gradual decline in locomotor behavior until death. In general, the temporary exacerbation of locomotor decrement on the day of irradiation in the combination group, was offset by the beneficial effects of the combined treatment on 30-day survival.

In addition to radioprotection by pharmacological agents, the immediate behavioral effects of radiation may be mitigated by material (e.g., lead shielding) placed between the radiation source and the subject. The use of shielding reportedly modifies radiation injury and enhances protection from lethality (Bohr et al., 1958; Taketa et al., 1959). Shielding studies designed to mitigate the behavioral effects have focused on either head shielding/body exposed or body shielding/head exposed. Several studies suggested that head shielding may offer protection from early performance decrement (Thorp et al., 1970; Thorp and Young, 1971). Early performance decrement is defined as an immediate (within 5-10 minutes) degradation in performance after exposure to a large, rapidly delivered dose of ionizing radiation. However, because the results from other shielding studies (see Mickley et al., 1989) do not confirm the previous findings, the role of shielding on early performance decrement is equivocal. This may be due to the use of a variety of performance models, radiation types, doses, and dose rates.

Using the accelerating rotorod (accelerod), we investigated the ability of lead shielding of the head and/or the body to mitigate radiation-induced motor deficits in Sprague-Dawley rats. In this behavioral paradigm, rats must maintain their balance for as long as possible on a rotating rod that accelerates at 1 rpm/second (Bogo et al., 1981). After a 3-week training period, the rats were exposed to 130 Gy ^{60}Co at 20 Gy/minute. This dose has been determined to be that at which 90% of rats in the accelerod model exhibit early performance decrement (Bogo et al., 1989). Early performance decrement was operationally defined as degradation of performance two z-scores (95%) below baseline levels at 10 minutes postirradiation. In addition to

testing at 10 minutes after irradiation, accelerod performance was evaluated at 15, 30, 60, and 120 minutes as well as at 24, 48, and 72 hours after irradiation. The experimental groups tested were (1) body shielded/head irradiated, (2) head shielded/body irradiated, (3) unshielded/head and body irradiated, and (4) sham-irradiated controls. At 10 minutes postirradiation, 8% of the rats in the head-shielded group exhibited early performance decrement, significantly fewer than the 67% in the body-shielded group or the 92% in the unshielded group. An analysis of variance of the behavioral tests conducted during the first 2 hours revealed that

... our studies demonstrate that motor function tests can be used to establish a sensitive index for determining the effects of radiation and radioprotectors on performance.

control rats performed significantly better than rats in all shielded conditions. Head-shielded rats performed significantly better than the body-shielded and unshielded rats. Analysis of the test periods from 24 to 72 hours indicated that, while the performance of the body-shielded rats was not significantly different from that of the controls, it

was significantly better than that of the head-shielded and unshielded rats.

These findings suggest that, as measured by accelerod performance, head shielding offers the greatest protection in the initial 2 hours of testing, which suggests mediation by the central nervous system. Body-shielded rats, however, performed better than body-irradiated rats, suggesting that the motor deficits observed on days 1-3 postirradiation may be due to gastrointestinal effects.

We also investigated the effects of radiation-induced fatigue, using rats trained to run on a treadmill (Dudley et al., 1982; McMaster and Carney, 1985). It is well established that humans exposed to sublethal levels of radiation exhibit signs of fatigue and weakness (Anno et al., 1989), which are likely to result in significant performance decrement. The rats were trained over a period of 5 weeks to maintain a running time of 20 minutes with the treadmill set at a 15-degree incline and a speed of 27 meters/minute. Rats were then either sham irradiated or exposed to a 7-Gy dose of ^{60}Co γ radiation delivered at 1 Gy/minute (N=12/group). After irradiation, rats were tested for running endurance on the treadmill 3 days/week for 39 days. Irradiated rats showed a significant decrease in body weight

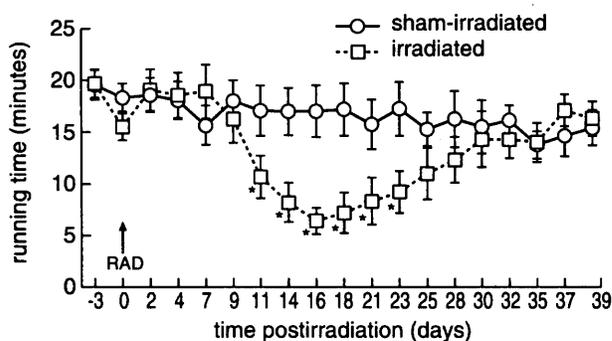


Fig. 4. The effects of 7 Gy ^{60}Co radiation on treadmill performance. The vertical bars represent the mean \pm SEM. Asterisks indicate $p < 0.05$ from the sham-irradiated control group.

and treadmill performance. Body weight was significantly decreased beginning on day 2 postirradiation and remained below control levels for the duration of the study. Irradiated rats, on days 11-23 postirradiation, exhibited a significant performance decrement of up to 62% compared with that of control rats (fig. 4). The treadmill performance of irradiated rats returned to control levels by day 25 postirradiation and remained there until the conclusion of the study. The data indicate that a sublethal dose of γ radiation can result in a transient degradation of performance of a physically demanding motor performance task.

Taken together, the results of these studies demonstrate that motor function tests can be used to establish a sensitive index for determining the effects of radiation and radioprotectors on performance.

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Casualty Management Program

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Program goals

- Design and evaluate therapeutic protocols for treatment of radiation damage to the hemopoietic and gastrointestinal organ systems.
- Reduce the periods of neutropenia and thrombocytopenia associated with recovery after high-dose radiation exposure while establishing long-term reconstitution of the hemopoietic system.
- Prevent development of the sepsis inflammatory response syndrome and septic shock by enhancement of the host's nonspecific cellular defense and/or by interruption of the pro-inflammatory cytokine cascade.
- Investigate the physiological and physical mechanisms responsible for evoking emesis and altered gastrointestinal motility to radiation in order to develop better treatment protocols.

Requirement

The battlefield radiation or nuclear disaster environment will likely be uncontrolled and ill defined. The radiation may differ between or among the environments in quality, energy, and dose rate. The exposure may be nonuniform and heterogeneous. In addition, the military and/or civilian physician will treat irradiated personnel who may also be traumatized with burns or wounds or both. At this time, injuries associated with midlethal doses in the hemopoietic range will likely be manageable. That is, the ill-defined and uncontrolled nature of radiation exposure in a nuclear accident usually forecasts a nonuniform exposure with variable dose distribution, which suggests a possible sparing of bone

marrow and/or gastrointestinal stem cells. With this in mind, it is possible that recovery may be induced after radiation exposure well into the hemopoietic syndrome range.

The overall objective of this program is to design effective therapeutic protocols for treatment of radiation damage to two major organ systems, the hemopoietic and gastrointestinal. Recovery from the lethal effects of irradiation across both the hemopoietic and intestinal syndromes requires at least three key events. The first is the self-renewal of several populations of pluripotential and multipotential stem cells, which will eventually lead to both short-term recovery from immediate radiation effects and long-term reconstitution of the hemopoietic system and intestinal mucosa. The second is the generation of functional cells; that is, of the neutrophils, platelets, and mucosal epithelial cells that will prevent the morbidity and mortality associated with the consequent hemorrhage, sepsis, and electrolyte imbalances. The third is the production of these functional cells within a critical, clinically manageable period of time defined by the capacity to support the irradiated host with antibiotics, platelet transfusions, and aggressive fluid and electrolyte therapy.

The military services require information regarding effective therapeutic protocols specific to enhancement of recovery from the acute effects of radiation. Immediate consequences of effective therapies are (1) the reduction of morbidity and mortality associated with marrow aplasia and consequent infection and hemorrhage as well as gastrointestinal symptoms and (2) significant reductions in term and cost of health care. It must be emphasized that the continued development of new generation therapies will depend upon an integrated research program stressing the interaction of ap-

plied and mechanistic approaches to the problems of deciphering the regulation of hemopoietic and gastrointestinal stem cell physiology.

Strategy

Five research study groups in two scientific departments address the research questions relevant

to the combined military requirements that shape the mission of AFRRI. Classic techniques and methodologies of experimental hematology and gastrointestinal physiology are integrated with newer approaches stressing molecular biology, recombinant cytokines, cellular cytokine receptors, and fluorescent intracellular probes. Study models range from the petri dish to the nonhuman primate.

Reconstitution of hemopoiesis and resistance to sepsis and septic shock in preclinical models of radiation-induced marrow aplasia and gram-negative infection

The efficacy of cytokine therapy and blockade of the inflammatory cascade

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Project 00082

This project aims to develop and evaluate therapeutic protocols that (1) will enhance recovery of hemopoietic stem and progenitor cells in sublethally and/or lethally irradiated animals so as to reduce the period of neutropenia and thrombocytopenia during long-term reconstitution of the hemopoietic system and (2) will enhance the functional activity of the host's nonspecific cellular defense and/or will interrupt the proinflammatory cytokine cascade in order to prevent development of sepsis and septic shock due to infection by opportunistic pathogens.

Recovery, across both the hemopoietic and intestinal systems, from the lethal effect of irradiation

requires at least three key events. First is the self-renewal of several populations of pluripotential and multipotential stem cells. That self-renewal will eventually lead to both a short-term recovery from immediate radiation effects and a long-term reconstitution of the hemopoietic system and intestinal mucosa. Second is the generation of functional end cells (neutrophil, platelet, and mucosal epithelial cells). Generation of those cells will prevent the morbidity and mortality associated with irradiation and the consequent hemorrhage, sepsis, and electrolyte imbalance. Third is the production of these functional cells within a critical, clinically manageable period of time defined by the support available to the irradiated host in terms of antibiotics, platelet

transfusions, and aggressive fluid and electrolyte therapy.

The cytokine network is the common denominator in the regeneration of hemopoietic stem cells, in the production of functional neutrophils and platelets, and in the initiation, progression, and control of the pathogenesis associated with infection, sepsis, and acute inflammation.

Gram-negative sepsis: Treatment with recombinant, species-specific cytokines

This report focuses on our efforts to prevent morbidity and mortality resulting from bacteria-induced gram-negative intraperitoneal sepsis. Our canine model of gram-negative sepsis provided an excellent opportunity to evaluate the role of lineage-specific cytokines in prophylactic and therapeutic treatment protocols.

The two primary cytokines that modulate the production and function of neutrophils are granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). These cytokines exert their proliferative effects through their action on bone-marrow-derived progenitor cells. An additional advantage with these cytokines is that specific receptors are found on all the differentiated cells of the granulocyte lineage. Exposure of neutrophils to these cytokines has shown that the cytokines can directly and indirectly modulate neutrophil function. For example, both GM-CSF and G-CSF can prime neutrophils for increased respiratory burst activity. This

capacity is associated with enhanced bactericidal activity.

Gram-negative sepsis is a primary cause of morbidity and mortality after irradiation, chemotherapy, and/or severe trauma. Even though neutrophils are the host's key cellular defense against invading pathogens, their release of toxic oxygen radicals may be detrimental to the host.

We evaluated the preclinical efficacy of recombinant canine G-CSF (rcG-CSF), administered in prophylactic and therapeutic protocols, for reducing morbidity and mortality in our canine model of gram-negative *Escherichia coli* peritoneal sepsis. Two questions were examined. The first was whether an rcG-CSF dose sufficient to increase polymorphonuclear (PMN) leukocytes to very high levels would increase host resistance to a lethal bacterial challenge or whether a lower rcG-CSF dose that would increase PMN leukocytes to a relatively lesser degree would prime them to be better functioning cells. The second question was whether a large increase in primed PMN leukocytes would cause significant damage to organs, such as the lungs, during the septic episode.

Figure 1 illustrates the experimental protocol used for these studies. On day 0, purpose-bred beagles, 9-12 kg, had *E. coli*, 0111, (15×10^9 organisms/kg) implanted intraperitoneally in a fibrin clot under general anesthesia. Animals were randomly assigned to receive one of three daily subcutaneous treatments, which began 9 days before clot placement and continued for 12 days. The treatments were (1) rcG-CSF, 5 $\mu\text{g}/\text{kg}$ mixed in Hanks' bal-

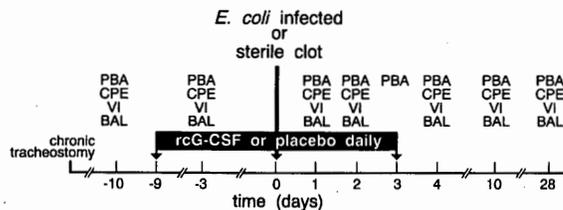


Fig. 1. Experimental design for prophylaxis and therapy with rcG-CSF in a canine peritoneal clot model of bacterial sepsis. Cardiopulmonary evaluations (CPEs), bronchoalveolar lavages (BALs) with alveolar cell analysis, and peripheral blood analyses (PBAs) were performed in all animals 10 and 3 days before as well as 1, 2, 4, 10, and 28 days after volume infusions (VIs). Starting on day 0, all animals were treated with ceftriaxon (100 mg/kg) once daily for 5 days and with Ringer's lactate solution (60 ml/kg) intravenously 2, 6, 24, and 28 hours after clot placement.

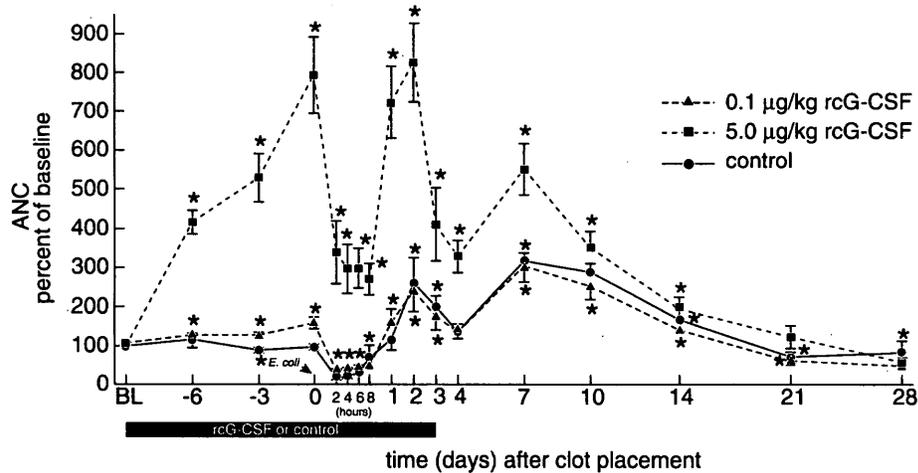


Fig. 2. Absolute neutrophil count (ANC) as a percent of baseline for canines administered rcG-CSF or a control protein and challenged with *E. coli* (15×10^9). *Significant difference ($p < 0.05$) from baseline values.

anced salt solution (HBSS); (2) rcG-CSF, 0.1 $\mu\text{g}/\text{kg}$ mixed in HBSS and 4.9 $\mu\text{g}/\text{kg}$ human serum albumin (HSA); and (3) HSA, 5 $\mu\text{g}/\text{kg}$ mixed in HBSS (controls).

At the high dose of 5.0 $\mu\text{g}/\text{kg}/\text{day}$, rcG-CSF significantly elevated peripheral PMN leukocytes to peak values 9 days after administration (the time of bacterial clot implantation) to 800% of baseline ($50\text{--}60 \times 10^3/\text{mm}^3$). The low-dose rcG-CSF increased PMN leukocytes to approximately 150% of baseline, a value less than that for the high dose but significantly greater than that for the control (fig. 2). The administration of rcG-CSF was continued for 3 consecutive days following bacterial clot placement.

The hallmark parameters of cardiovascular dysfunction, mean arterial blood pressure and left ventricular ejection fraction (LVEF), decreased significantly from baseline as evidence of development of severe hypotension and septic shock in all three groups (fig. 3). Both parameters reached nadirs at day 2 and recovered at the same rate in all three treatment groups. A noted exception was that, compared with controls, the high-dose animals had significantly less ($p < 0.05$) depression of LVEF at day 1 after infected clot placement.

One consideration was the large number of primed PMN leukocytes found circulating in the high-dose animals. Would these PMN leukocytes be attracted to sensitive organs such as the lung? The results from broncho-alveolar lavages (BALs)

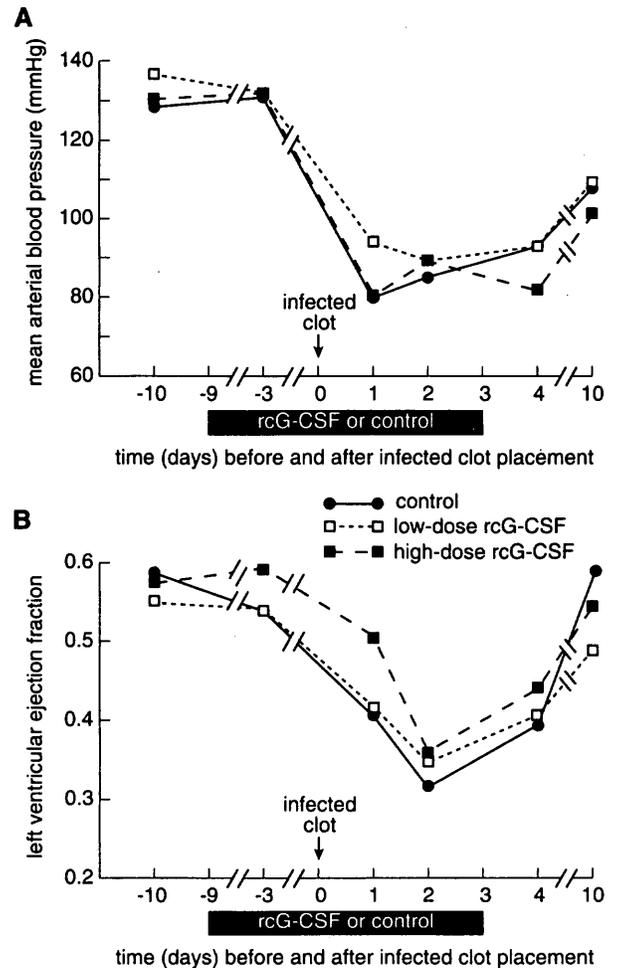
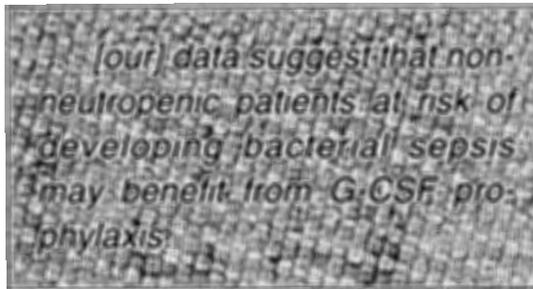


Fig. 3. Cardiovascular dysfunction, in terms of (A) mean arterial blood pressure and (B) left ventricular ejection fraction, after infected clot placement.

performed in these animals are summarized, with respect to neutrophils, in figure 4. An early but significant rise in BAL PMN leukocytes was noted for the high-dose animals. This rise was not sustained and remained within the increased number of BAL

PMN leukocytes found in the control and low-dose animals. Overall, PMN leukocytes increased in the BAL during the septic episode, but the administration of rcG-CSF at high and low doses neither influenced their migration into lung tissue nor had any adverse effect on lung function as determined by the mean alveolar to arterial oxygen gradient.

Lethality was significantly reduced ($p < 0.05$) in the high-dose animals. The control and low-dose animals had 40% and 41.2% lethality, respectively, while the high-dose animals showed 11.7% lethality. It is also of interest that circulating endotoxin measured significantly lower in the high-dose animals. Treatment with rcG-CSF may increase levels of circulating endotoxin inhibitors such as lipopolysaccharide-binding protein or bacteria-permeability-increasing protein. In these animals, rcG-CSF may also affect the pathogenesis of sepsis by inducing the release of inhibitors or blockers of proinflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF). The increased synthesis and release of IL-1 receptor antagonist and/or the shedding of soluble receptors for the



cytokines IL-1 and TNF, may act to modulate the inflammatory response and result in a favorable outcome. The ability of rcG-CSF to augment host defenses by such mechanisms as recruitment and priming of neutrophils, in addition to those possibilities mentioned

above, may all have contributed to this favorable outcome. Applied clinically, these data suggest that non-neutropenic patients at risk of developing bacterial sepsis may benefit from G-CSF prophylaxis.

Radiobiology of the gastrointestinal system

The goal of this research is to evaluate radiation-induced injury to the intestinal mucosa, specifically, the renewal cells of the crypt and the functional cells of the villus. Irradiation destroys dividing cells preferentially and, if the cells are not replaced, leads to diminution of the intestinal mucosa. Radiation-induced injuries to the small intestine have been shown to release factors (cytokines) that are responsible for alterations in the intestinal mucosa and cell renewal.

A radiation injury model for the small intestine of a Sprague-Dawley rat has been established to investigate the influence of partial intestinal irradiation on the epithelial cells of shielded and irradiated regions. These studies examine the mechanism

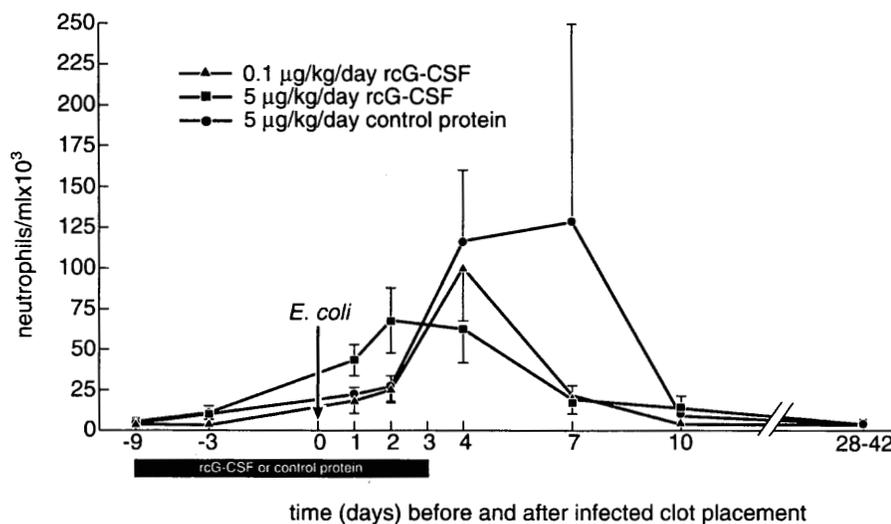


Fig. 4. Neutrophils in bronchoalveolar lavage. An early rise in BAL PMN leukocytes in high-dose animals was not sustained.

for the interaction of cytokines with injury and recovery in the intestinal epithelial cells.

In this model, the exteriorized intestine is irradiated, and the body of the rat is shielded. Shielding, which enables us to study all or part of the irradiated small intestine without irradiating large volumes of bone marrow, produces longer survival times after lethal irradiation. With shielding, 20 Gy of radiation induces intestinal death in 8-10 days in contrast to 4-7 days without shielding.

Irradiation of the exteriorized intestine produces the intestinal syndrome while preserving the hemopoietic cells in the bone marrow and eliminating the influence of irradiated bone marrow on this syndrome and survival. This permits evaluation of therapy for the small intestine in a model system not compromised by neutropenia. Agents that can lessen the clinical symptoms or enhance mucosal recovery can be tested with this model.

The histology of the intestinal mucosa of irradiated and shielded tissues can be influenced by the region of the intestine irradiated. Irradiation of the upper half of the small intestine resulted in a more pronounced effect on the villus and an earlier death than irradiation of the lower half, which resulted in an enhanced response in the crypt and a later death. This suggests that stimulatory or inhibitory factors released after irradiation can affect renewal and functional intestinal epithelial cells.

The crypt stem cell of the intestinal mucosa is a primary target for radiation injury. In these experiments, we determined the labeling index of intestinal crypts after entire or partial intestinal irradiation to evaluate whether radiation can modify intestinal cells in shielded intestine. Eight-week-old Sprague-Dawley rats were anesthetized and, with aseptic surgical procedures, the small intestine of each was exteriorized to (1) the entire 80 cm, (2) the proximal 40-cm region, or (3) the distal 40-cm region. The gut regions were exposed to 20-Gy photon radiation with a bremsstrahlung beam generated from a linear accelerator operated at 18 MVp with an average photon energy of 6 MeV and a dose rate of 10 Gy/minute. After irradiation, the intestine of each

was returned to the abdominal cavity, muscles were sutured, and the skin was surgically stapled. All shielded animals died. Those in which the entire 80 cm of small intestine or the proximal 40-cm region was irradiated had a median survival of 10 days after irradiation; those in which the distal 40-cm region was irradiated died 12 days or later after irradiation.

Histological study of the labeling index of crypts 5 days after small intestinal irradiation is presented in table 1.

Table 1. Labeling index at day 5 after 20-Gy photon irradiation.

Intestinal region	Normal intestine	Irradiated intestine	Shielded intestine
40-cm proximal	29%	8% ^a	36% ^b
40-cm distal	27%	6% ^a	21% ^b

^a $p < 0.01$ compared to normal, N=6 rats, 15-20 crypts per rat.

^b $p < 0.05$ compared to normal, N=6 rats, 15-20 crypts per rat.

These studies show that labeling of epithelial cells in the intestinal crypts depends on the region of the small intestine irradiated. Irradiation of the proximal intestine resulted in a decrease in the labeling index of the crypts in the shielded distal small intestine. In contrast, irradiation of the distal intestine resulted in an increase in the labeling index of crypts of the shielded proximal intestine.

In summary, small-intestine exteriorized loops that receive a single high dose of radiation can influence labeling of crypts in other regions. One explanation is that humoral factors released after irradiation can modify the epithelial cells of the intestinal mucosa. The identity of the factors is unknown; however, they can be stimulatory or inhibitory to epithelial cells.

Our goal is to identify and administer specific agents or growth factors and determine end points (including the appearance of diarrhea, alterations in mucosal histology, and survival time) in order to elucidate mechanisms of some of the radiation-induced complex physiological interactions in the intestinal mucosa.

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Evaluation of cytokine-stem cell interactions in determining post-irradiation hemopoietic regeneration

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Project 00132

It is well known that radiation exposure can predispose a host to life-threatening infections and hemorrhage (Hammond et al., 1954; Benacerraf, 1960; Bond et al., 1965). These consequences typically have been attributed to an inability to generate new mature white blood cells and platelets subsequent to depletion of extremely radiosensitive hemopoietic stem cells (Till and

McCulloch, 1961; Carsten et al., 1976; Millard and Blackett, 1981).

Recent advances in the identification of previously elusive hemopoietic stem cells (Magli et al., 1982; Spangrude et al., 1988; Spangrude, 1989; Ploemacher and Brons, 1989; Jones et al., 1990) have allowed more precise evaluations of their radiosensitivity, revealing these cells to be more radioresistant than formerly believed (Meijne et al., 1990, 1991). Thus, lethality from radiation-induced marrow aplasia may, more likely, be a consequence of the inability of surviving hemopoietic stem cells to self-renew and differentiate fast enough to produce the cells necessary to prevent sepsis and hemorrhage. Since stem cell proliferation and differentiation are regulated by endogenously produced cytokines (Metcalf, 1985; Quesenberry et al., 1989; Moore, 1991), we hypothesize that radiation-induced disruption of the production of critical endogenous hemopoietic cytokines interferes with the ability of surviving stem and progenitor cells to repopulate the irradiated host in a timely manner.

The ultimate goal of this research project is to elucidate breakthrough treatments for radiation-induced bone marrow aplasia by identifying endogenous cytokine profiles needed to selectively stimulate the proliferation and differentiation of specific stem and progenitor cells critical for hemopoietic regeneration. Such therapies should benefit individuals exposed to high-dose exposures during radiation accidents, clean-up operations, solar flares in space, radiotherapy, and radiomimetic chemotherapy.

We have two major objectives. One is to characterize the ability of various cytokines to regulate specific stem and progenitor cells *in vivo* and to use this information to selectively design and time cytokine treatments to accelerate multilineage hemopoietic regeneration and increase survival following radiation exposure. The other objective is to characterize the effects of radiation on the production of endogenous hemopoietic cytokines in order to correlate endogenous cytokine changes with hemopoietic regeneration following sublethal radiation exposure.

To characterize which stem, progenitor, and mature cells are regulated by specific cytokines when administered *in vivo*, we have used a high sublethal *in vivo* radiation model established in B6D2F1 mice. Previously we had demonstrated the

ability of G-CSF (granulocyte colony-stimulating factor) therapy to significantly accelerate postirradiation hemopoietic regeneration in this model (Patchen et al., 1990). In fiscal year 1992, we examined the functional activity of the phagocytic cells generated in irradiated G-CSF-treated mice. Phagocytic activity was based on oxidative burst potential induced by opsonized zymosan in both unprimed and C5a-primed bone marrow cells. A chemiluminescent technique was used to measure oxidative activity. Following 17 days of G-CSF treatment, bone marrow and splenic myeloid cells in irradiated mice exhibited not only greater oxidative activity than cells from saline-treated irradiated mice but also greater oxidative activity than cells from non-irradiated controls (fig. 1).

Additionally, new cytokines and cytokine combinations were evaluated for the treatment of radiation-induced hemopoietic injury in 7.75-Gy irradiated B6D2F1 mice. These included c-kit ligand, which is also known as stem cell factor and mast cell growth factor (MGF), as well as granulo-

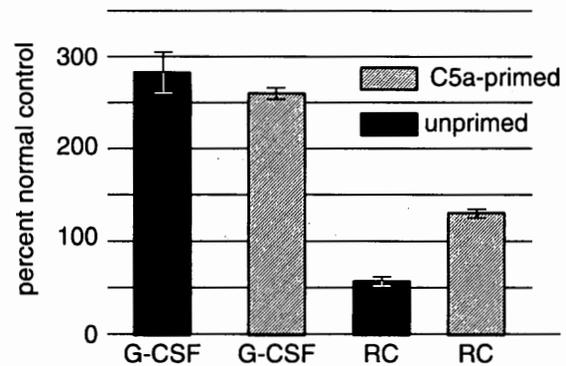


Fig. 1. Effect of daily G-CSF treatment (100 µg/kg/day subcutaneously) on oxidative burst activity of regenerating bone marrow cells in B6D2F1 mice 17 days after a 7.75-Gy ⁶⁰Co exposure. Results represent the mean ± standard error of four experiments. RC is radiation control cells.

cyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and combinations of these cytokines (fig. 2). Based on spleen colony-forming unit (CFU-s) and granulocyte-

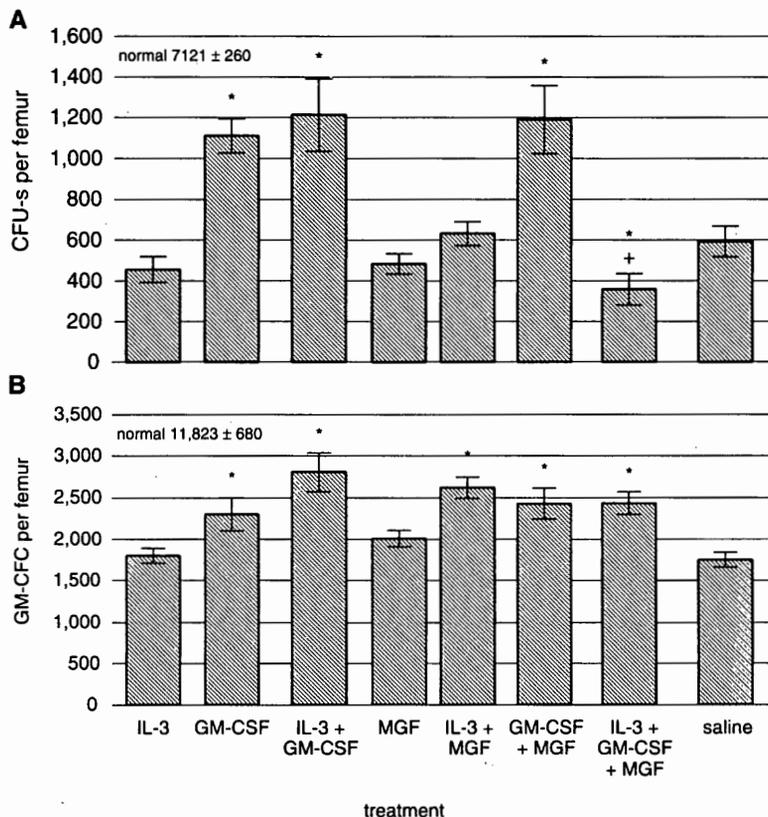


Fig. 2. Effects of MGF, GM-CSF, IL-3, and combinations of these cytokines (each at 100 µg/kg/day subcutaneously) on bone marrow (A) CFU-s and (B) GM-CFC recovery in B6D2F1 mice 17 days after a 7.75-Gy ⁶⁰Co exposure. An * represents significant differences (p<0.05) from saline values; + represents significant differences (p<0.05) from GM-CSF values.

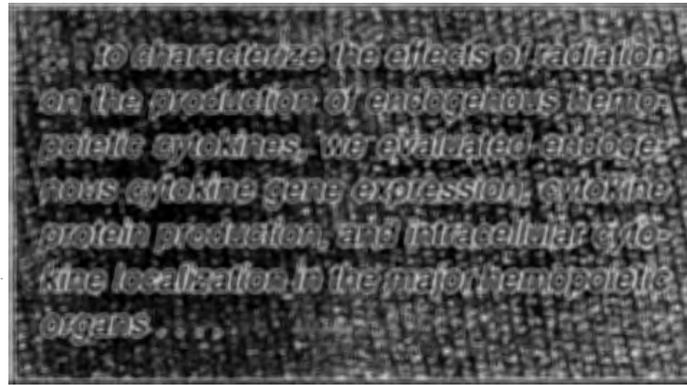
colony-forming cell (GM-CFC) recoveries, GM-CSF accelerated hemopoietic recovery whereas IL-3 or MGF alone had only a marginal effect. The combination of IL-3 and GM-CSF produced better recovery than GM-CSF alone. Surprisingly, however, when c-kit ligand was administered in combination with GM-CSF or GM-CSF plus IL-3, hemopoietic regeneration either was not further enhanced or was down-regulated. These effects are contrary to previously published *in vitro* effects of MGF and indicate the necessity of evaluating agents in appropriate *in vivo* animal models.

To accomplish our other objective — to characterize the effects of radiation on the production of endogenous hemopoietic cytokines — we evaluated endogenous cytokine gene expression, cytokine protein production, and intracellular cytokine localization in the major hemopoietic organs (the bone marrow and the spleen) of female B6D2F1 mice exposed to a severe myeloablative, but sublethal, 7.75-Gy dose of ^{60}Co radiation.

Technology developments related to gene expression studies

Initial studies performed in fiscal year 1991 revealed that total ribonucleic acid (RNA) yields obtained from bone marrow and splenic tissues in irradiated mice were too low (1-10 μg) to allow quantitation of gene expression by traditional Northern analysis. Because of this, sensitive reverse transcriptase polymerase chain reaction (RT-PCR) methods were established not only to detect but also to quantitate cytokine gene expression in tissues obtained from irradiated mice. The quantitative ability of the RT-PCR detection system has been further established in our lab in fiscal year 1992.

Using multiple aliquots of a single RNA sample, we demonstrated that the RT-PCR technique is precise, yielding an average variation of less than 15%. Accurate RNA quantitation, however, was found to depend on multiple factors, including amplification cycle number. Optimal cycle numbers



have been worked out for IL-1, IL-3, IL-6, tumor necrosis factor (TNF), GM-CSF, and c-kit ligand.

Interestingly, when we attempted to internally control amplifications by coamplifying a test gene and a constitutive gene, the results differed markedly from those for single gene amplifications (correlation coefficient 0.0024), negating such a procedure as an internal control. Furthermore, we found that GAPDH (glyceraldehyde phosphate dehydrogenase) expression, a constitutive gene that is often used as a control gene in molecular biology studies, was also altered following radiation exposure, complicating its use as a control gene.

In fiscal year 1992, using our improved techniques to detect and quantitate cytokine gene expression following radiation exposure, we have evaluated radiation-induced effects on the expression and production of the hemopoietic cytokines c-kit ligand, GM-CSF, IL-1, and TNF.

C-kit ligand

It has been suggested that c-kit ligand, a cytokine produced by bone marrow and splenic stromal cells, plays a pivotal role in hemopoietic regulation. *In vitro*, c-kit ligand has been shown to synergistically enhance the proliferative effects of multiple hemopoietic cytokines, including IL-1, IL-3, IL-6, G-CSF, GM-CSF, and erythropoietin. *In vivo*, c-kit ligand has been shown to partially reverse the hemopoietic defects observed in Steel mice. However, the role of this cytokine in postirradiation hemopoiesis *in vivo* remains unclear.

Recent data suggest that the ability of IL-1 to enhance survival when administered prior to irradiation may be due in part to the induction of c-kit ligand (Neta et al., 1993); however, when c-kit ligand has been directly administered to mice following radiation exposure, only marginal hemopoietic effects have been observed (Patchen et al., 1993).

The purpose of this study was to investigate c-kit ligand gene expression in the bone marrow and spleen following a severe myeloablative radiation exposure. Bone marrow and spleen cells were isolated from normal control mice and from mice exposed to 7.75 Gy of ^{60}Co on days 1, 2, 3, 4, 7, 10, 14, 17, 21, and 28 postirradiation. Total RNA was isolated and reversely transcribed into cDNA; the cDNA was amplified with PCR primers specific for murine c-kit ligand by the RT-PCR technique. Amplified cDNAs were electrophoresed and blotted onto a nylon membrane; cytokine transcripts were identified by hybridization with labeled c-kit ligand probe. Subsequent autoradiographs were quantitated with a scanning densitometer. Amplification of individual normal bone marrow and spleen RNA revealed basal c-kit ligand expression in all animals. Following irradiation, c-kit ligand expression was elevated for approximately 2 weeks.

Further experiments quantified differences in c-kit ligand gene expression on day 2 postirradiation, which in preliminary studies appeared to be a peak time of expression. At day 2 postirradiation, bone marrow c-kit ligand expression was tenfold greater than normal levels and splenic c-kit ligand expression was approximately threefold greater than normal levels (fig. 3).

GM-CSF

In vitro and in vivo studies have shown that the cytokine GM-CSF dramatically affects hemopoietic proliferation, differentiation, and function. In

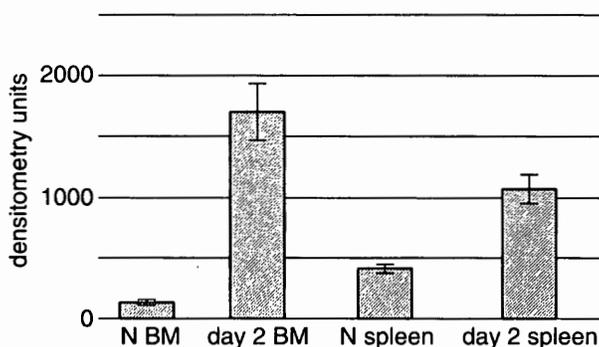


Fig. 3. Bone marrow (BM) and splenic c-kit ligand gene expression in normal (N) B6D2F1 mice and in mice on day 2 following a 7.75-Gy ^{60}Co exposure. Results represent the mean \pm standard error of 4-6 separate experiments and were corrected for GAPDH expression.

fiscal year 1991, we reported that GM-CSF gene expression was increased for at least 10 days following a 7.75-Gy radiation exposure such as that used for c-kit ligand studies described above. In fiscal year 1992, we also verified increased endogenous GM-CSF protein production as measured by ELISA on days 1 and 4 postirradiation, which represented times of moderately increased and dramatically increased gene expression, respectively. Compared with that in nonirradiated controls, GM-CSF protein in irradiated mice was increased in both the bone marrow and spleen. In bone marrow, increased levels were observed only on day 1 postirradiation; in the spleen, increased levels were evident on days 1 and 4 (fig. 4). Although splenic GM-CSF gene expression appeared to parallel splenic protein production, bone marrow correlations were less clear-cut (fig. 4). Explanations for this discrepancy are under investigation.

IL-1

The cytokine IL-1 plays a role in hemopoietic regulation by functioning as a stimulatory cofactor in the presence of other cytokines. In fiscal year 1991, we reported that following a 7.75-Gy radiation exposure, increased expression of IL-1 could be detected in murine bone marrow and spleen for approximately 2 weeks. In fiscal year 1992, we used this cytokine to investigate whether radiation dose-related changes occur in cytokine expression and

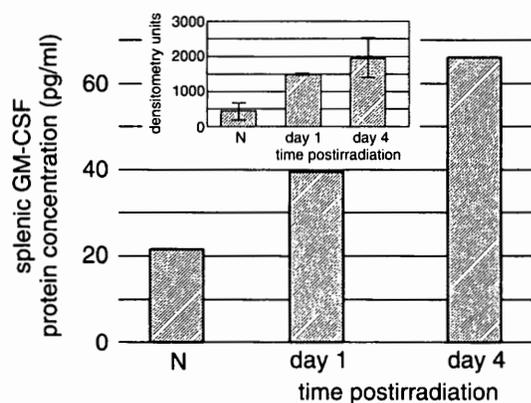


Fig. 4. Splenic GM-CSF protein production and gene expression (insert) in nonirradiated (N) B6D2F1 mice and in mice on days 1 and 4 following a 7.75-Gy ^{60}Co radiation exposure. Protein was determined by ELISA (one experiment). Gene expression results represent the mean \pm standard error of 2 experiments.

production. Specifically, we compared the effects of sublethal and lethal ^{60}Co exposures on IL-1 gene expression and protein production.

The sublethal dose used for these studies was the 7.75 Gy used in our previous gene expression studies. Although this dose is extremely myeloablative, all mice hematopoietically recover and survive the exposure. The lethal radiation dose was 9.75 Gy, which is lethal for 100% of the mice within 30 days postexposure. In these studies, we evaluated IL-1 effects only in the spleen because it is easier to obtain sufficient RNA and cell yields from this organ than from bone marrow. Eight hours after sublethal and lethal radiation exposures, splenic cellularities were reduced, respectively, to 34% and 32% of normal and, 24 hours afterward, to 12% and 9%.

Our initial studies established the patterns of gene expression within the first 24 hours postexposure. Sublethal and lethal doses induced similar time-dependent changes in IL-1 gene expression: At 5 minutes postexposure, gene expression was undetectable (less than normal); at 2 hours postexposure, it had returned to normal ranges; and at subsequent postexposure times (4-24 hours), it was greater than normal. The intensity of these effects appeared to be dependent on radiation dose (fig. 5).

To determine whether IL-1 cytokine production was also increased, we used an ELISA technique to quantitate splenic IL-1 protein. At 5 minutes postexposure, the IL-1 protein levels in the sublethally and lethally irradiated mice were not significantly different from the level in the control mice. However, by 8 hours postexposure, the levels in irradiated mice were significantly elevated.

TNF

TNF is a cytokine known to possess diverse biological activities. While other cytokines evalu-



ated in our studies tend to stimulate hemopoiesis, TNF has been demonstrated to inhibit it. Furthermore, this cytokine may play a critical role in generalized tissue injury that occurs following radiation exposure.

In fiscal year 1991, we demonstrated that, following a 7.75-Gy exposure, TNF message expression as well as protein production were increased in bone marrow and spleen. In addition to methods to detect protein in cell lysates, we have been developing methods to detect cytokine protein in situ.

Using electron microscopy and sophisticated immunohistochemistry techniques developed previously in our laboratory (Chock and Schmauder-Chock, 1990; Chock et al., 1991), we have expanded our efforts to identify and localize TNF in tissues of mice following a 7.75-Gy radiation exposure. As early as 2 hours following radiation exposure, TNF could be localized in various cells within the hemopoietic and related systems. In par-

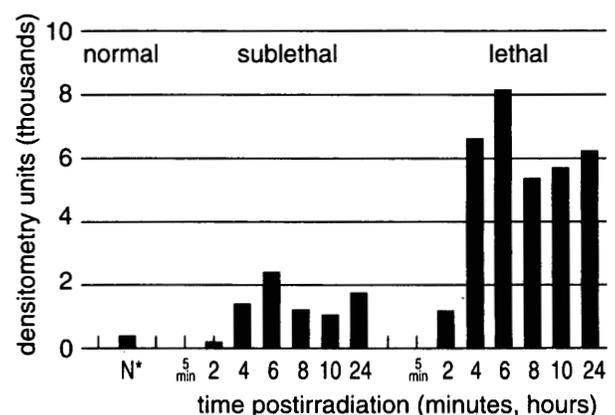


Fig. 5. Splenic IL-1 gene expression in nonirradiated B6D2F1 mice and in mice within the first 24 hours following a sublethal (7.75-Gy) or lethal (9.75-Gy) ^{60}Co exposure.

ticular, the secretory granules of granulocytes in arterial walls stained heavily for TNF (fig. 6).

Cell separation studies related to gene expression

We know that a variety of cells within the hemopoietic microenvironment produce hemopoietic cytokines. Some of these cells, such as macrophages, are known to be extremely radioresistant (Schmidtke and Dixon, 1972; Geiger and Gallily, 1974) and, therefore, may play a critical role post-irradiation in producing cytokines that affect hemopoietic regeneration. Lymphocytes, on the other hand, have typically received little consideration in

early hemopoietic recovery due to their extreme radiosensitivity. Even within a given cell type, radiosensitivities vary as shown by our previous description of a radioresistant subpopulation of CD4+ T-lymphocytes in the spleen and thymus.

It is also apparent that different cell types possess distinct but overlapping repertoires of cytokine expression capabilities. For example, both macrophages and T-lymphocytes produce IL-6, G-CSF, M-CSF, GM-CSF, and TNF. But of these two cell types, only the macrophages produce IL-1, and only the T-lymphocytes produce IL-3.

Our TNF studies demonstrated that in situ detection of cytokine protein could facilitate the iden-

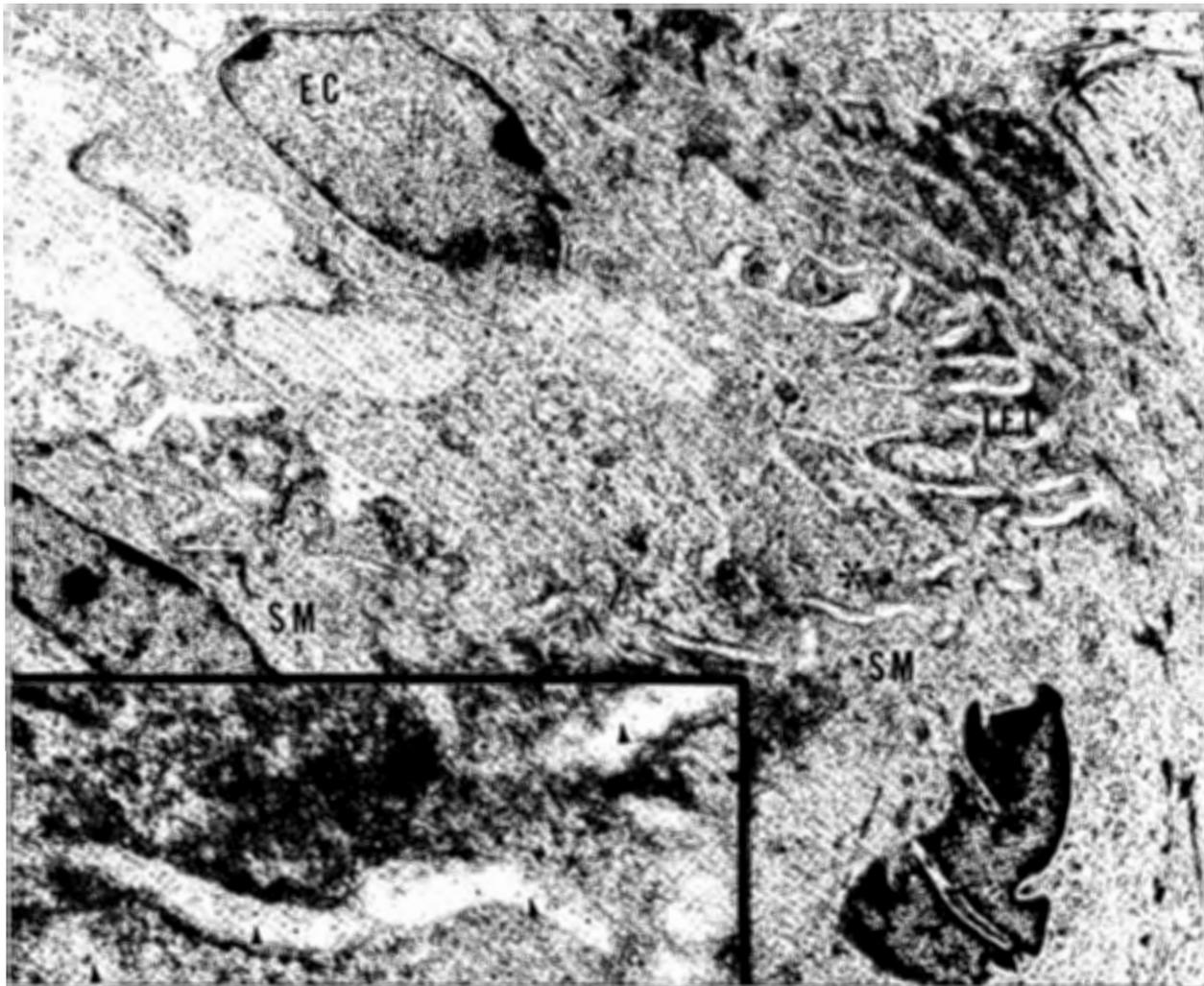


Fig. 6. In situ detection of TNF α in the internal elastic lamina (IEL) of a mouse arteriole 2 hours after a 7.75-Gy ^{60}Co radiation exposure. TNF α is probably secreted by smooth muscle (SM) cells. Endothelial cells (EC) are attached to the arteriole lining adjacent to the IEL. The insert represents a high magnification image of the IEL (*), where TNF α label can be visualized (arrowheads).

tification of cell types that play a role in cytokine production. We would also like to be able to detect gene expression *in situ* at the molecular level. Although this is theoretically possible with *in situ* hybridization, most laboratories, including ours, have not been extremely successful in implementing these difficult and time-consuming techniques.

As an alternate approach to examining which cells produce specific cytokines at particular times following radiation exposure, techniques to isolate purified populations of a single cell type were established. Using fluorescence-activated cell sorting techniques, we isolated CD4+ T-lymphocytes at 95% purity (fig. 7). Techniques are being developed to purify other cell types, particularly splenic macrophages, which may also greatly influence hemo-

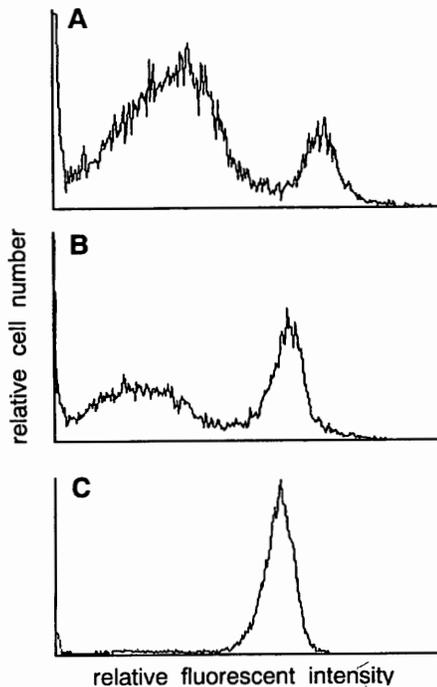


Fig. 7. Purification of CD4+ spleen cells from normal B6D2F1 mice. (A) Single cell suspensions were prepared from the spleen, treated with NH_4Cl to lyse erythrocytes, stained with phycoerythrin-conjugated anti-CD4 monoclonal antibody, and analyzed by flow cytometry; CD4+ cells = 13.4%. (B) B-lymphocytes were removed by incubation of the cell suspension with magnetic beads coated with sheep anti-mouse IgG and removal of the cell/bead rosettes with a magnet. Cells were stained with anti-CD4 and analyzed as above; CD4+ cells = 34%. (C) Cells remaining in the suspension were stained with anti-CD4 monoclonal antibody, sorted by fluorescence-activated cell sorting techniques, and analyzed for purity; CD4+ cells = 94.6%.

poiesis by cytokine production. Once sufficient quantities of purified cells are isolated, their ability to express and produce cytokines will be determined as previously described for bone marrow and spleen cell suspensions.

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Survival and management of sepsis in mouse models of injury found in radiation disasters

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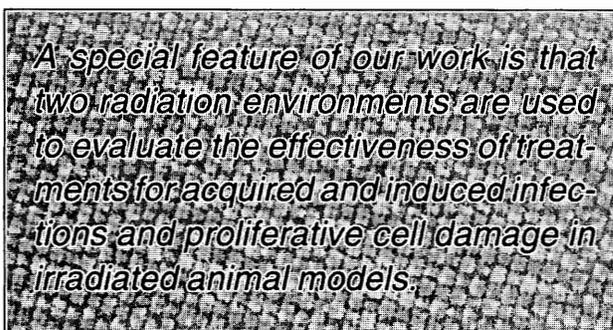
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The goal of our research is to identify key injuries to nonspecific antibacterial defenses caused by radiations found in nuclear reactor accidents or nuclear weapons detonation environments. We aim to develop effective treatments against or therapies for acquired opportunistic bacterial infection or induced bacterial infections in animal models of such radiation situations and in irradiated animals inflicted with additional tissue trauma. Infections in irradiated individuals inflicted with additional trauma (wounds and

burns) are more difficult to treat than infections due to radiation exposure alone.

The nonspecific host defense system is generally composed of tissues that contain actively dividing cells that are susceptible to damage caused by ionizing radiation. Thus, we focus our efforts on the protection, repair, and recovery of the hemopoietic, gastrointestinal, and skin proliferative cell systems in animals through the use of biological response modifiers and antimicro-



A special feature of our work is that two radiation environments are used to evaluate the effectiveness of treatments for acquired and induced infections and proliferative cell damage in irradiated animal models.

bial substances with and without radioprotective chemicals.

A special feature of our work is that two radiation environments are used to evaluate the effectiveness of treatments for acquired and induced infections and proliferative cell damage in irradiated animal models. These radiation environments are that of (1) pure gamma (γ) photons produced by the AFRRI standard ^{60}Co source and (2) a mixed field of neutrons (n) and γ photons, both of which have mixed energy levels as produced by the AFRRI TRIGA reactor. In TRIGA reactor irradiations of mice, refined dosimetry techniques are provided by AFRRI's Radiation Biophysics Department in conjunction with the National Institute of Standards and Technology. The techniques, which employ magnesium foils, indicate an $n/(n+\gamma)$ proportion of 0.71. The field used in this report is identical to that previously described (Ledney et al., 1991) wherein an $n/(n+\gamma)$ ratio of 1 was reported.

In the report on AFRRI research during fiscal year 1991, we noted that effective therapy for acquired infection in $n/(n+\gamma)=0.71$ -irradiated animals is more difficult to achieve than in γ -photon-irradiated animals. This is particularly the case when wounds are inflicted after irradiation. Because of this, in a first series of experiments, mice were treated intraperitoneally (i.p.) with a low dose (200 mg/kg; 25% of the lethal dose, LD_{10}) of the radioprotective chemical WR-151327 prior to irradiation and as an adjunct to the use of biological response modifier or antimicrobial therapy. WR-151327 was selected because at high doses it is effective against neutron damage to the hemopoietic system (Steel et al., 1987), has an apparent protective effect against radiation damage to the gastrointestinal system (Sigestad et al., 1986), and increases survival of γ -photon-irradiated mice when given orally (Steel-Goodwin et al., 1992). Further, performance decrements (locomotor activity) induced by low doses of WR compounds are reversible by caffeine treatments (Landauer et al., 1992).

The radiation doses used (10.25-Gy γ photons and 5.6-Gy $n/(n+\gamma)=0.71$) are comparable in that (1) 30-day mortality is about 80% and (2) the survival times of the decedents are the same after either quality and dose of radiation (12 ± 2 days). These data support the idea that mortality after these radiation doses is related to hemopoietic failure. The relative biological effectiveness as measured at the $\text{LD}_{50/30}$ is 1.8 for the $n/(n+\gamma)=0.71$ -irradiated mice

compared to the γ -photon-irradiated mice. In radiation dose response studies, we determined that the dose reduction factor (DRF) for γ -photon-irradiated, WR-treated mice was 1.53 ($\text{LD}_{50/30}$ of WR-treated mice was 13.78 Gy and of γ -photon-irradiated mice was 9.03 Gy). The DRF for $n/(n+\gamma)=0.71$ -irradiated mice was 1.30 ($\text{LD}_{50/30}$ of WR-treated mice was 6.44 Gy and of those given only $n/(n+\gamma)=0.71$ was 4.98 Gy).

We are investigating the protective factors for this WR compound dose in animals inflicted with wound trauma shortly after exposure to each radiation environment. In addition, since personnel may be exposed to radiation more than one time in the performance of duties (e.g., during spaceflight), we will examine the residual injury induced by the mixed field radiations and sustained in hemopoietic cell compartments of WR-protected and unprotected mice.

Presented in figure 1 are the 180-day survival percentages of mice treated i.p., before or after irradiation, with 8 mg/kg (200 $\mu\text{g}/\text{mouse}$) of the biological response modifier synthetic trehalose dicorynomycolate (S-TDCM). The figure also shows the survival of animals given 40 mg/kg of the antimicrobial L-ofloxacin orally at 24-hour intervals starting 3 days after irradiation and ending 21

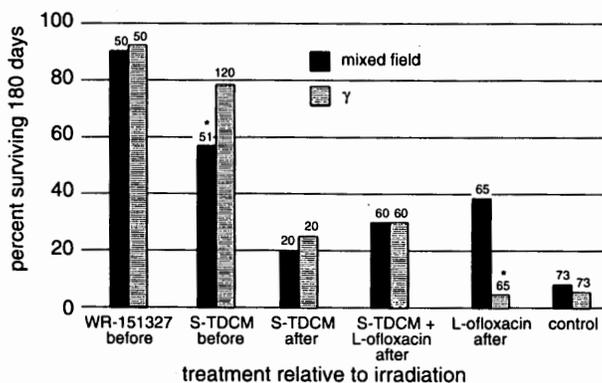


Fig. 1. Incidence of survival in B6D2F1/J mice 180 days after 5.6-Gy mixed field $n/(n+\gamma)=0.71$ or 10.25-Gy γ -photon irradiation and treatments with WR-151327, S-TDCM, or L-ofloxacin. Statistical differences were determined by χ^2 -square analysis (*= $p<0.01$). All treatments, except L-ofloxacin therapy, of γ -photon-irradiated mice increased survival as compared with saline treatment of controls ($p<0.01$). All treatments, except S-TDCM therapy, of $n/(n+\gamma)=0.71$ -irradiated mice increased survival as compared with saline treatment of controls ($p<0.01$). Numbers at the top of the bars indicate the total number of mice in each experiment.

days later. We previously reported on the rates for 30-day survival from acquired infection in irradiated animals treated with S-TDCM (Ledney et al., 1991) or L-ofloxacin (Brook and Elliott, 1991) as well as on the rates for survival from *Klebsiella pneumoniae*- and *Pseudomonas aeruginosa*-induced infection in irradiated mice (Brook and Ledney, 1992). Interestingly, L-ofloxacin increased the survival percentage of $n/(n+\gamma)=0.71$ -irradiated mice but not of γ -photon-irradiated mice. In current studies, we are attempting to understand why an antimicrobial effective against gram-negative bacterial infection is effective in mixed-field-irradiated mice and not in γ -photon-irradiated mice.

In future experiments, we intend to use the data obtained for WR-151327, S-TDCM, and L-ofloxacin to develop treatments that enhance resistance to bacterial infection in combined injured hosts.

Biological response modifiers are agents that alter the relationship between a disease and a host by changing the host's biological response to the disease. We have shown that S-TDCM, which is derived from bacterial cell walls (Ledney et al., 1992; Madonna et al., 1991), and ImuVert® (IMV), which contains bacterial membrane vesicles and ribosomes, improve survival of septic γ -photon-irradiated mice. However, bacterial lipopolysaccharide (LPS) and monophosphoryl lipid-A (MPL), which is a nontoxic derivative of LPS, do not.

In collaborative studies with investigators at the Uniformed Services University of the Health Sciences and the University of Colorado Health Sciences Center, we determined, by reverse transcription-polymerase chain reaction (Svetic et al., 1992), the genetic expression of cytokines as a measure of the biological response of mice to S-TDCM and IMV. We hypothesized that these substances increase survival in bacteria-challenged, γ -photon-irradiated mice by affecting the production of cytokines.

The genetic expressions of interleukin-1 β , interleukin-6 (IL-6), and granulocyte-colony stimulating factor were stimulated strongly by IMV, but not by S-TDCM, LPS, or MPL, on days 5 and 7 after sublethal irradiation followed by challenge with

Klebsiella pneumoniae on day 4. IMV and S-TDCM may increase survival of septic, irradiated mice by different mechanisms.

Survival following irradiation with LD_{100/30} doses is based on recovery of impaired hemopoietic function. Our previous studies using antibodies to the cytokines IL-1R, tumor necrosis factor (TNF), and IL-6 demonstrated that endogenous production of these three biological response modifiers is required for untreated mice as well as mice protected with LPS, IL-1, or TNF to survive lethal irradiation (Neta et al., 1991, 1992a). Presently, we show that anti-c-kit ligand/steel factor (SIF) antibody similarly abrogates LPS- and IL-1-induced radioprotection (Neta

et al., in press). Furthermore, administration of this antibody to unmanipulated mice increased LD_{50/30} radiation lethality from 50% to 100%. Such an effect was not obtained with anti-IL-3, anti-IL-4, or anti-granulocyte-macrophage colony-stimulating factor antibody. Thus, like IL-1, TNF, and IL-6, SIF is required for survival after lethal irradiation. Our future studies will explore the most recently observed synergistic effects of combinations of IL-1 and SIF and mechanisms underlying this synergy.

The radioprotective cytokines induced by biological response modifiers and released during inflammation also have important endocrine effects, in particular, on the hypothalamic-pituitary-adrenal (H-P-A) axis (Perlstein et al., 1991). We continued our studies of these effects and found that IL-1 and IL-6, TNF and IL-1, TNF and IL-6 interact synergistically in activating the H-P-A axis (Perlstein et al., 1991; Neta et al., 1992a) and that IL-6 is obligatory in the induction of adrenocorticotrophic hormone by IL-1 (Neta et al., 1992b). In addition, employing electron microscopic immuno-cytochemical techniques, we reported that IL-1 activates both vasopressin-deficient and vasopressin-containing, corticotropin-releasing hormone axons in rats (Whitnall et al., 1992a; Whitnall et al., 1992b). We plan to clarify how cytokines mediate the activation of the H-P-A axis by endotoxin/lipopolysaccharide. In addition, we will document the acute H-P-A response to radiation injury and determine if the H-P-A response to cytokine injection is altered by radiation.

Our future studies will explore the most recently observed synergistic effects of combinations of IL-1 and SIF [anti-c-kit ligand/steel factor]

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Mature cell dysfunction due to ionizing radiation

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The goals of this project are to understand the functions of cells that are involved in host defense and trauma-induced repair and to examine the effects of radiation and/or radioprotectors on those cells. The project focuses on macrophages and endothelial cells and examines (1) the essential roles they play in fighting life-threatening infections and maintaining barrier functions, (2) the processes that activate the cells, and (3) the processes that allow pathogens to enter them. Previous studies from our laboratory and others have indicated that radiation produces functional changes in endothelial cells and macrophages and that those changes may modify host defense and tissue repair (Gallin and Green, 1987; Haimovitz-Friedman et al., 1991).

Our studies on the macrophage, a pivotal cell in host defense, have focused on the effects of radiation on ionic mechanisms involved in cellular homeostasis and signal transduction during cytokine-initiated activation. In the characterization of pH regulation in the J774 macrophage-like cell line, we have examined several hydrogen extrusion processes (McKinney and Moran, 1992). These in-

clude (1) a vacuolar-type H⁺-ATPase that contributes significantly to maintaining resting intracellular pH but contributes only minimally to recovery from an intracellular acid load and (2) a Na⁺/H⁺ exchanger that is activated during intracellular acid load. We assessed the activity of these transport systems in irradiated, lipopolysaccharide-stimulated, and control J774 cells by monitoring intracellular pH using the fluorescent dye BCECF and following extracellular pH changes produced by H⁺ extrusion in weakly buffered cell suspensions. In these cells, interestingly, both radiation exposure and lipopolysaccharide treatment induced an intracellular acidification that increased with time after treatment (see table 1), suggesting that pH homeostatic processes are changed following radiation exposure.

In order to assess changes in ionic conductances following exposure to radioprotective cytokines, we have adapted the perforated patch clamp technique to the study of macrophages. This technique allows membrane currents to be monitored without the loss of intracellular constituents (>200D) that may be important in cellular signaling cascades. Studies using this technique have demonstrated that the electrophysiological properties of the macrophage, in contrast to those of the neuronal cells, are resistant to exogenously added H₂O₂. That is, H₂O₂ up to 15 mM had no effect on the resting membrane potential, leak conductance, or voltage-dependent inwardly rectifying K⁺ conductance in macrophages (Judge and Gallin, in press).

As part of our investigations of homeostatic ionic mechanisms important in macrophage sur-

Table 1. Long-term effect of exposure to bacterial lipopolysaccharide (LPS) or ionizing radiation on resting pHi of J774 macrophages.

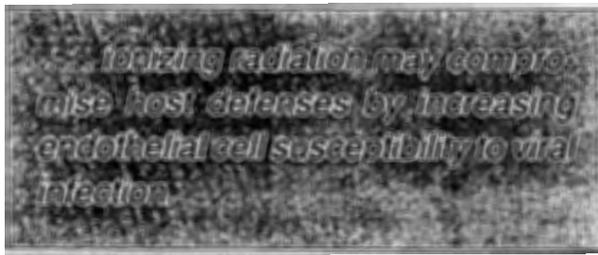
Days post-exposure	Δ pHi after LPS	Δ pHi after radiation
0	-0.02 ± 0.09 (7)	-0.09 0.04 (3)
1	-0.17 ± 0.05* (10)	-0.04 0.03 (4)
2	-0.12 ± 0.05* (6)	-0.37 0.03* (4)
3	-0.23 ± 0.02* (6)	-0.19 0.03* (4)
4		-0.33 0.02* (4)

LPS: 10 mg/ml

Ionizing radiation: 20 Gy at 1 Gy/minute

*Significant difference from pHi of control cells

(~7.5) measured the same day. N values are in parentheses.



vival and activation, a characterization of regulatory volume decreases (RVDs) was completed in two macrophage-like cell lines, THP-1 cells and HL-60 cells. These studies demonstrated that the activation of a quinine-inhibitable K^+ permeability and a 3,5-diiodosalicylic-acid-inhibitable chloride permeability during cell swelling resulted in an RVD (Gallin and Mason, 1992). Fura-2 measurements of intracellular calcium levels indicate that, while intracellular calcium increases during swelling, macrophage volume regulation does not require activation of calcium-activated conductances; RVDs occur in the absence of extracellular calcium and in BAPTA-loaded cells that have reduced intracellular calcium levels. These results indicate that the K^+ conductance responsible for volume regulation is likely to be a novel conductance not yet characterized electrophysiologically.

Vascular endothelial cells play important roles in preventing the leakage of critical plasma components, mediating the migration of leukocytes into tissue, regulating blood clotting and immune responses, and repairing wounds. Our studies on endothelial cells have focused on (1) characterization of radiation-induced endothelial cell injury and death, which can lead to plasma leakage, and (2) examination of the effects of combined radiation and viral infection injury on cell survival and leukocyte-endothelial cell adhesion, a critical first step in the recruitment of macrophages to sites of infection.

Our studies indicate that, in endothelial cells, radiation results in both apoptotic cell death and necrotic cell death. Apoptosis is often referred to as programmed cell death since it is defined by a series of morphological and biochemical events that are often dependent on continued protein synthesis. Apoptotic cells die in a controlled fashion without the inflammatory reactions induced by cell debris released during necrotic cell death. We have used the DNA fragmentation, which characteristically occurs during apoptosis, to quantitate radiation-induced changes in bovine aortic endothelial cells

(BAECs). BAECs exposed to gamma (γ) radiation exhibited a time- and dose-dependent increase in the number of apoptotic cells. At 24 hours after exposure to 20 Gy, $17 \pm 1\%$ (mean \pm standard error, or SE) of DNA in irradiated BAECs was fragmented, compared with $3 \pm 1\%$ of that in control BAECs (fig. 1). By 48 hours, the fragmentation in exposed BAECs had decreased to $7 \pm 2\%$, suggesting that apoptosis may be an acute, transient response to ionizing radiation. All the fragmented DNA were present in BAECs that had detached from the culture dish, thus providing an easily separable population of apoptotic BAECs for future biochemical analyses. Future studies will examine the role of growth factors in protecting endothelial cells and the responsiveness of the surviving (nonapoptotic) endothelial cells to these growth factors.

We studied leukocyte-endothelial cell adhesion, using the HL-60 myeloid cell line and cultured human umbilical vein endothelial cells (HUVECs). In contrast to the observations of Dunn et al. (1986), undifferentiated HL-60 cell adherence to endothelial cells exposed to γ irradiation (1-10 Gy) was not changed during the period of 4 to 72 hours postirradiation. However, influenza-virus-induced HL-60 adherence was enhanced in a dose-dependent manner by prior radiation exposure (Colden-Stanfield et al., 1992). This synergistic effect of virus infection and irradiation on leukocyte-endothelial adhesion was confirmed with cAMP-differentiated HL-60 cells as well as human neutrophils. The increased adherence in virus-infected irradiated endothelial cell monolayers was also associated with an increase in the expression of the viral protein hemag-

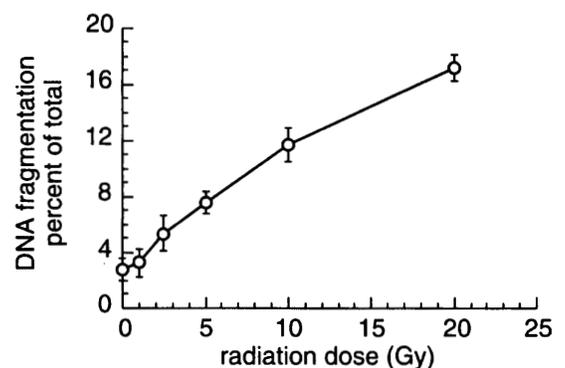


Fig. 1. Apoptosis in BAECs. DNA fragmentation, a measure of the extent of apoptosis, was quantitated in BAECs 24 hours after exposure to γ radiation (1 Gy/minute). The fragmented DNA is expressed as a percent of the total DNA in the sample. Each data point is the mean \pm SE of three experiments.

glutinin (HA) antigen (fig. 2). Furthermore dose responses of the cytotoxic effect of viral infection, which we assessed by monitoring lactic dehydrogenase release, indicated that prior exposure to radiation significantly enhances influenza-induced cytotoxicity (fig. 3). Thus, ionizing radiation may compromise host defenses by increasing endothelial cell susceptibility to viral infection.

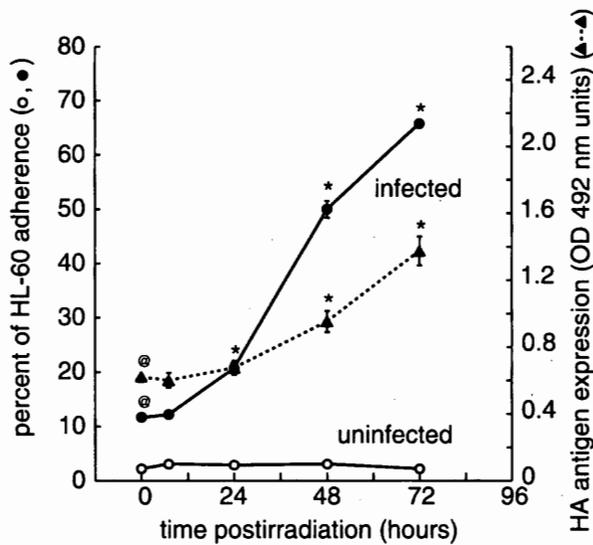


Fig. 2. Effect of 10-Gy γ irradiation on HL-60 adherence to and hemagglutinin (HA) antigen expression on uninfected and influenza-virus-infected HUVEC monolayers. Measurements began at 7 hours postinfection. $\textcircled{\bullet}$ Infected, sham-irradiated group versus uninfected, sham-irradiated group ($p < 0.05$). * Infected, irradiated group versus infected, sham-irradiated group ($p < 0.05$).

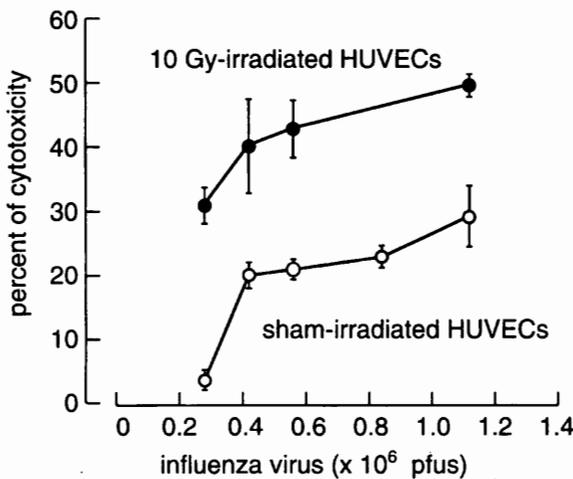


Fig. 3. Effect of 10-Gy γ irradiation on the induction of cytotoxicity by influenza virus 30 hours after infection of HUVEC monolayers and 52 hours postirradiation.

In related studies of virus-cell interactions, the influenza virus/human red blood cell model system has been used to examine membrane fusion, the earliest stage of virus infection. Fluorescent video microscopy methods have been developed to observe for the first time the movements of all the major viral molecular components, lipids, proteins, and RNA during fusion of "live" viral particles (Lowy et al., 1990). The experiments confirmed that flu is capable of fusion at 16°C , well below physiological temperatures. Further experiments comparing fusion at 37°C and 16°C showed that, contrary to generalized views, viral proteins and lipids enter the cell at different rates; lipids diffuse more rapidly than envelop proteins. Surprisingly, RNA appears to enter before the dissolution of the viral envelop proteins. These studies provide the basis for examination of the effects of radiation on viral-membrane fusion.

Since cell pathogens not only fuse directly with plasma membranes but also can exploit other endocytic processes to gain entry into cells (Marsh and Helenius, 1989), we examined the effects of radiation on fluid phase endocytosis. The goal of this portion of the project is to determine if radiation and/or free radicals alter endocytic processes and, thus, make cells more susceptible to pathogens. By monitoring the uptake in CV-1 cells of lucifer yellow, a marker of fluid phase endocytosis, we demonstrated that fluid phase endocytosis was decreased following irradiation. Uptake followed for 5-120 minutes and, as shown in figure 4, was significantly decreased in CV-1 cells exposed to 20 Gy.

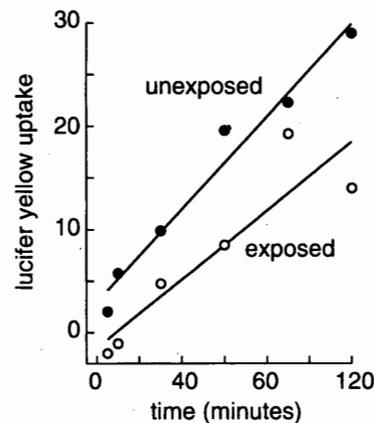


Fig. 4. Effect of radiation on fluid phase endocytosis in CV-1 cells. Results are from a representative experiment for γ radiation exposure of 20 Gy at 1 Gy/minute. Both exposed and control cell values have been corrected for nonspecific binding of lucifer yellow.

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Effects of ionizing radiation on gastrointestinal physiology

Emesis and fluid and electrolyte loss

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Project 00107

Our purpose is to develop treatments for radiation-induced gastrointestinal injury. The symptoms of radiation-induced gastrointestinal dysfunction depend on the dose (fig. 1). At low

doses (1 Gy), prodromal symptoms consist of vomiting, gastric stasis, and diarrhea. At higher doses (7.5 Gy), prodromal symptoms are followed by alterations in cellular transport processes and intes-

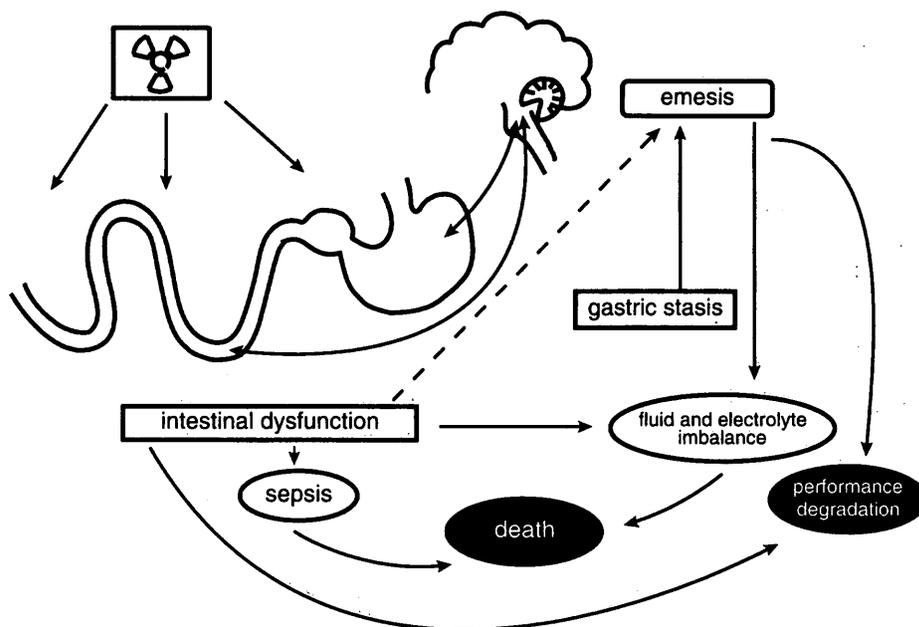


Fig. 1. Effects of irradiation on gastrointestinal physiology that lead to performance degradation or death.

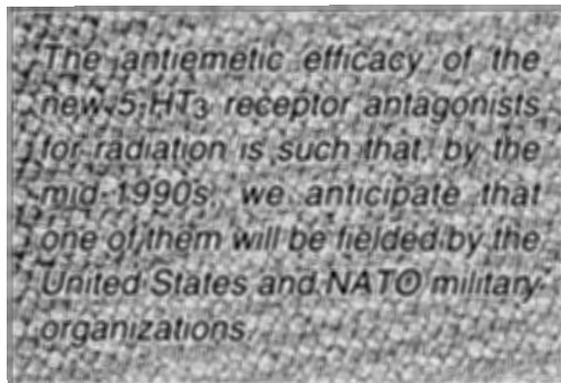
tinal stem cell proliferation, resulting in severe fluid and electrolyte loss and infection. These latter effects contribute to death associated with the gastrointestinal syndrome (Quastler, 1956).

The exact mechanisms underlying these symptomatology are not completely understood. Both the central nervous system and the gastrointestinal system have been proposed to initiate the prodromal processes. For the gastrointestinal syndrome, the dividing cells of the small intestine are the critical radiation target. However, it is evident that other factors are involved as well. Thus, an immediate goal of the project is to understand the mechanisms underlying radiation's effects on gastrointestinal function. In this vein, our studies focus on those areas that may contribute to the postirradiation symptomatology (gastrointestinal motility, gastric infection, gastrointestinal transport). These studies use a variety of animal and cell models and both in vitro and in vivo techniques.

The prodromal symptoms may be caused by the release of an unknown gastrointestinal substance (cf. King and Makale, 1991). Controversy exists as to whether this substance triggers emesis by acting locally on sensory afferent endings of the peripheral autonomic nervous system (neural hypothesis) or by circulating to the brain stem area postrema (humoral hypothesis) (Harding, 1988). Our studies have added data in support of the neural hypothesis. Vagotomies and peripherally acting serotonin subtype three (5-hydroxytryptamine; 5-HT₃) receptor antagonists significantly attenuate radiation-induced nausea and vomiting (cf. King and Makale, 1991) in animal models. Such results have led to a double-blind clinical study of a 5-HT₃ receptor antagonist in patients subjected to total-body irradiation in preparation for bone marrow transplants at Georgetown University.

The antiemetic efficacy of the new 5-HT₃ receptor antagonists for radiation is such that, by the mid-1990s, we anticipate that one of them will be fielded by the United States and NATO military organizations. One in-depth investigation of these

compounds and radiation-induced emesis addressed whether the 5-HT₃ receptor antagonists were effective antiemetics for other types of radiation, especially neutrons. Neutrons have a greater relative biological effectiveness (RBE) on the gastrointestinal syndrome itself (Broerse, 1968, 1975; Hornsey and Vatistas, 1968; Sigdestad and Scott, 1972; Broerse and Zoetelief, 1984; Sigdestad et al., 1986).



Rabin and King (1992) reported in preliminary studies that two 5-HT₃ receptor antagonists, eusatron and ondansetron, were effective antiemetics for a 2-Gy mixed neutron/gamma (γ) radiation (midline neutron dose: total dose ratio = 0.86). Furthermore, in a separate group of animals, prior subdiaphragmatic vagotomy reduced the incidence and latency of retching in ferrets exposed to such a radiation field. These data are consistent with the idea that similar vagal and 5-HT₃-dependent mechanisms mediate emesis produced by exposure to both γ and neutron radiation.

These data are consistent with the idea that similar vagal and 5-HT₃-dependent mechanisms mediate emesis produced by exposure to both γ and neutron radiation.

In a second study, preliminary information (McClellan et al., 1992) shows that data from the ferret model of radiation-induced emesis can, with some adjustment in the time scale of the response, be used to predict the human emetic response. As an example, such a model predicted that ferrets, when given fractionated radiation doses, would show decrementing emetic responses to each fractional dose. That prediction was borne out.

To gain more information about peripheral mechanisms of radiation-induced emesis, we used a model to examine emesis to oral CuSO₄, a gastric irritant that acts via afferent neural pathways. We found that sectioning of both the vagal and splanchnic afferent pathways was required to abolish the emetic response (Makale and King, 1992). However, sectioning of the nerves, alone or in combination, did not abolish the concomitant elevated arterial pressure that accompanies the emetic events. Conversely, autonomic ganglionic receptor antagonists abolished the elevated arterial pressure but not the emetic events. That these two events can

be dissociated demonstrates that, despite their temporal proximity, they do not represent central nervous system activation of identical brain stem areas.

Another area of investigation concerns the mechanisms of emesis to the 5-HT₃ receptor antagonist zacopride. We had previously shown that the emetic response to an oral preparation of this compound was abolished by prior bilateral subdiaphragmatic vagotomy as well as by several pharmacologically distinct compounds including a 5-HT₃ receptor agonist, a cholinergic receptor antagonist, and a dopaminergic receptor antagonist (King, 1990). Others have shown that the emesis was due to the S(-) enantiomer of the previously used racemic mixture of zacopride (Sancilio et al., 1990; Middlefell and Price, 1991). In a preliminary study, we found that, using agonists of the aforementioned receptor ligands as well as an opioid receptor antagonist (naloxone), we could potentiate these emetic responses (King and Weatherspoon, 1992). While it is not yet known at what anatomical site these ligands could be acting, the data show that other receptor systems can modulate this emetic response.

Another gastrointestinal effect of radiation is suppression of gastric emptying of both solids (fig. 2) and liquids for up to 6 hours (Dubois et al., 1984; Dorval et al., 1985). Interestingly, gastric emptying is accelerated 7 days after irradiation, at the time diarrhea is observed.

As a result of these changes in gastric emptying, oral bioavailability of an antiemetic postirradiation may be modified. The antiemetic 5-HT₃ receptor

antagonist zacopride did not significantly modify gastric emptying in the control state or after irradiation (Dubois et al., 1988). Therefore, it is necessary to develop a gastrokinetic agent capable of preventing the suppression of gastric emptying that exists immediately after irradiation. The mediator responsible for this symptom of radiation sickness has not been identified, but the gastrointestinal hormone cholecystokinin (CCK) causes vomiting, slows gastric emptying, and suppresses appetite. These three signs and symptoms represent the hallmark of radiation-induced prodromal syndrome.

We previously demonstrated that, in primates, CCK plays an important role in the regulation of gastric motility after fatty meals; but its effect appears negligible after protein and carbohydrate meals. Recently, we investigated the possibility that radiation-induced emesis and suppression of gastric motility was caused by an endogenous release of CCK. We administered mixed solid/liquid meals tagged with ^{99m}Tc sulphur-colloid and ¹¹¹In-DTPA to four rhesus monkeys and measured gastric emptying using scintigraphy. We concurrently studied gastric motility by performing computer analysis of the epigastric electrogastrogram (EGG), a locally developed noninvasive method, which is similar to the cardiologist's electrocardiogram. We determined plasma CCK before and after irradiation, at various times after emesis, and 30, 60, and 90 minutes after the meals. Following exposure to 8-Gy total-body ⁶⁰Co γ irradiation (8 Gy/minute), retching occurred in four of four monkeys, and 2-15 emeses were observed in three of four monkeys. Emptying of solids and liquids was completely abolished, and gastric frequency

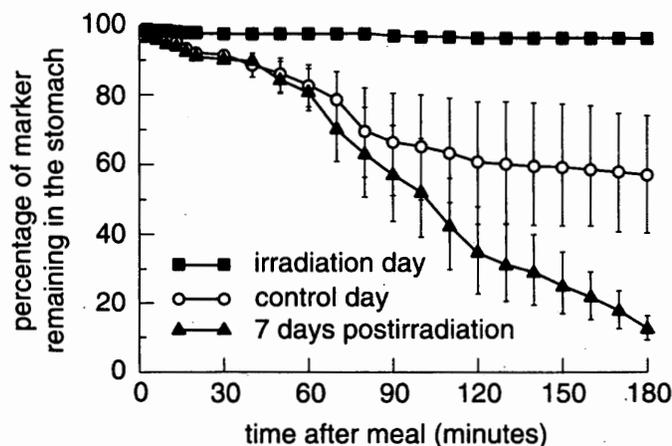
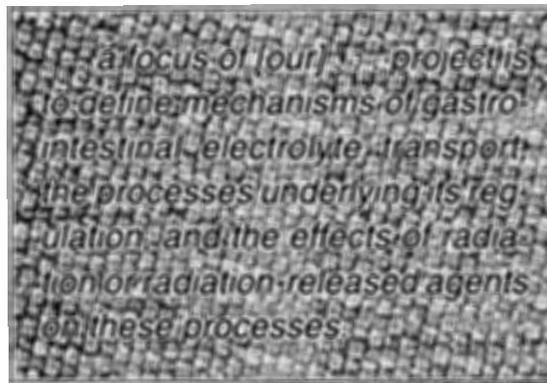


Fig. 2. Gastric emptying of solids in the monkey is suppressed on the day of irradiation and enhanced 7 days afterward.

significantly increased. Fasting plasma CCK remained unchanged 10 minutes after irradiation, increased after emesis, and had returned to baseline 15 minutes later. The CCK response to the meal was not different from that on control days, but none of the monkeys ate when returned to their cages.



To further study the role of CCK release in the emetic and gastric response to irradiation, we administered the specific CCK-A antagonist MK-329 before irradiation. Three of four monkeys that received MK-329 experienced retching and emesis within 30 minutes of irradiation. Gastric emptying and the EGG were not different from that in monkeys receiving irradiation plus vehicle, but three of four ate when returned to their cages 4 hours after irradiation. One week after irradiation, gastric emptying was accelerated, compared with preirradiated control values; and the postprandial plasma CCK response was significantly greater than before irradiation, when emptying was slower. Thus, CCK-A receptors may contribute to radiation-induced anorexia although they do not appear to play a role in retching, emesis, and the suppression of gastric emptying that is observed immediately after irradiation.

In addition to these potential peptidergic mediators, gastric infection may be involved in the gastric response to irradiation. Clinical studies suggest that *Helicobacter pylori* is responsible for unexplained vomiting in humans (Mohhudin et al., 1988) and for duodenal ulcers, gastritis, hypochlorhydria (Goodwin et al., 1986), cancer (Parsonnet et al., 1991), and possibly suppression of gastric emptying.

We observed small, curved, rod-shaped bacteria, measuring 3-4 μm long and 0.5-1.0 μm wide, in the proximity of mucosal epithelial cells in 8 of 29 monkeys. These bacteria were similar in appearance to *H. pylori* observed in humans (Newell et al., 1987; Dubois et al., 1991). However, as in humans, the frequency and route of transmission of *H. pylori* like organism (HPLO) infection in captive and free-ranging rhesus monkeys is unknown.

To study the seroepidemiology of this spontaneous gastric infection, rhesus monkeys were studied from three types of housing: indoor gang cages, outdoor corrals, and a free-range forest. Plasma immunoglobulin G (IgG) values were determined for all groups, and the cutoff IgG value for HPLO positivity was determined from a previous study. The present results demonstrate that 1-year-old rhesus monkeys of both sexes in all housing conditions have the lowest rate of infection. In addition, females of all age classes tend to have higher rates of infection than males, and males older than 11 years have the lowest rate of infection of all adults. Thus transmission of *H. pylori* occurs before 1 year and further increases with age. In addition, transmission appears to be unrelated to contact with humans and tends to occur less frequently in males of all ages in all housing conditions possibly because they have fewer social interactions than females. Finally, the lower rate of infection in older males may reflect a cohort effect or a decline in prevalence possibly due to gastric atrophy. The observations made in this animal model of human infection may have important implications for the understanding of the epidemiology of *H. pylori*.

A gastrointestinal syndrome characterized by fluid and electrolyte loss follows the prodromal phase of radiation-induced gastrointestinal dysfunction. Earlier studies demonstrated a dose- and time-dependent change in transcellular intestinal electrolyte transport by rabbit ileum before morphological changes occurred (Gunter-Smith, 1986). These studies suggested that, in addition to affecting mucosal integrity, irradiation affects cellular electrolyte transport processes. As a result, a focus of the project is to define mechanisms of gastrointestinal electrolyte transport, the processes underlying its regulation, and the effects of radiation or radiation-released agents on these processes.

We used the guinea pig gallbladder epithelium as a model system for the mammalian small intestine. This tissue is morphologically simple, possesses transport processes similar to those of the mammalian small intestine, and easily lends itself to electrophysiological techniques. One goal of our

studies has been to determine the physiologic role of a luminal membrane potassium conductance that we previously characterized (Gunter-Smith, 1988). This conductance (designated $gK_{TEA,CHTX}$) is voltage-dependent, Ca^{2+} -activated, and blocked by the potassium channel blockers tetraethylammonium (TEA), barium (Ba^{2+}), and Charybdotoxin (CHTX). Using transbladder ^{86}Rb or ^{42}K fluxes under short-circuit current conditions, we demonstrated that $gK_{TEA,CHTX}$ did not underlie prostaglandin E_2 -stimulated K secretion (Touzeau and Gunter-Smith, 1991). An additional uncharacterized K transport pathway was activated by prostaglandin E_2 .

Prostaglandin E_2 's stimulation of electrolyte secretion occurs primarily through cAMP-dependent K efflux pathways. However, in many cells, secretion is also elicited by Ca^{2+} -dependent pathways. Thus, we evaluated the role of $gK_{TEA,CHTX}$ in Ca^{2+} -stimulated K secretion from the effects of TEA and CHTX on ionomycin-stimulated K secretion. Ionomycin is a Ca^{2+} ionophore that has been shown to increase intracellular Ca^{2+} in a wide variety of cell types.

As shown in figure 3, both TEA and CHTX blocked ionomycin-stimulated K efflux. This result suggests that $gK_{TEA,CHTX}$ plays a role in secretion

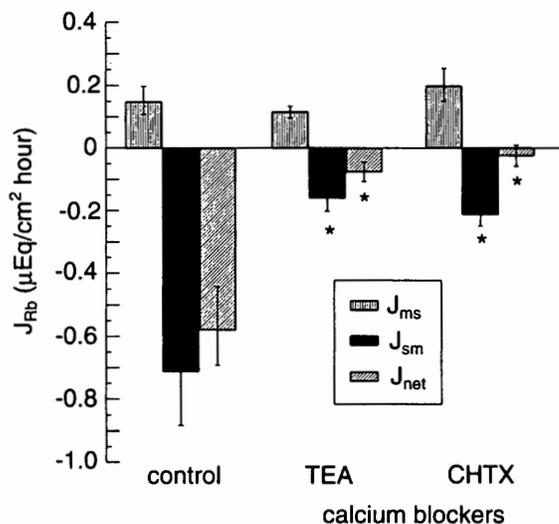


Fig. 3. Potassium efflux (J) carried by Rubidium (J_{Rb}) and stimulated by ionomycin in cultured gallbladder epithelium is blocked by two calcium blockers, TEA (tetraethylammonium) and CHTX (Charybdotoxin). J_{ms} is flux from mucosa to serosa. J_{sm} is flux from serosa to mucosa. J_{net} is net flux. An asterisk indicates a significant difference ($p < 0.05$).

elicited by Ca^{2+} -dependent but not cAMP-dependent secretagogues. Further, the results demonstrate the presence of at least two K efflux pathways, which are activated by different intracellular second messengers.

In an effort to develop therapies for radiation-induced diarrhea, we evaluated the efficacy of loperamide (the active ingredient of several over-the-counter antidiarrheal agents) in ameliorating the effect of irradiation on electrolyte transport by rabbit ileum. We compared the effect of loperamide (10^{-4} M) on the short-circuit current (I_{sc}) of ileal segments isolated from control and irradiated (10 Gy) animals 24 hours postirradiation. The short-circuit current is an *in vitro* measurement of active transcellular ion transport.

We had previously demonstrated that irradiation increases I_{sc} , reflecting an increase in electrolyte secretion (Gunter-Smith, 1986). Loperamide significantly decreased the I_{sc} in both control and irradiated segments, having a greater effect on irradiated segments. We are currently evaluating the effect of loperamide on transepithelial ileal fluxes of Na and Cl to determine if loperamide reverses the effect of radiation on these ion fluxes.

In addition to altering ion transport mechanisms of intestinal epithelial cells, irradiation blocks proliferation of intestinal stem cells in the crypts. The mucosa becomes denuded in 4-7 days, and the breakdown of the mucosal barrier contributes to bacterial infection and further fluid and electrolyte loss. The prostaglandins and their analogues alone or in combination with phosphorothioate derivations such as WR-2721 have been shown to protect intestinal stem cells from the injurious effects of irradiation on proliferation (Hanson and Thomas, 1983; Hanson, 1987). We are presently conducting studies to determine if radioprotection of the intestinal stem cell confers functional protection of the intestinal mucosa as well.

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Radiation Protection Program

Edward Clark, Ph.D., Radiation Biochemistry Department

Program goals

- Screen and identify potential pharmaceuticals that, when used in combination, provide a margin of safety against exposure to radiation.
- Determine the radiation-induced damage to DNA and the agents that will retard or prevent such damage.
- Use recent advances in molecular biology and recombinant DNA technology to enhance cellular repair processes.

Requirement

The possibility of uncontrolled radiation exposure exists, for example, at the site of a nuclear

accident or in an extraterrestrial space environment. Hence, pharmaceuticals that can help protect stem cells are essential in providing personnel an increased tolerance to radiation exposure.

Strategy

This program has three major goals. The first is to identify combinations of agents that provide some protection against ionizing radiation damage without adversely affecting performance. The second and third goals are aimed at developing a mechanistic approach to identify critical damage sites and to develop genetic repair strategies. Our working hypothesis is that variations in the structure or function of genes that regulate repair can significantly alter biological resistance to radiation.

Physiological mechanisms of radioprotection by combined agents

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Project 00162

Our objective is to develop radioprotectors that are useful in various radiation exposure scenarios and that produce minimal behavioral decrement and side effects. We propose that such agents may include phosphorothioates, immunomodulators, prostaglandins, prostacyclins, and nutritional factors (e.g., vitamins and minerals). We hypothesize that agents with different mechanisms of action, when combined, will maximize the radioprotective effect and minimize toxicity (Weiss et al., 1990).

Compared with other classes of radioprotectors, phosphorothioates, such as S-2-(3-aminopropylamino)ethyl phosphorothioic acid (WR-2721), are more effective against lethality in

mice (Weiss et al., 1990) and against radiation-induced late effects (i.e., mutagenesis and carcinogenesis). See Grdina et al., 1991.

Antioxidant nutrients like vitamin E were found to enhance radioprotection by a phosphorothioate. The increase in dose reduction factor (DRF) when both vitamin E and phosphorothioate were administered may indicate a way by which a less than toxic dose of phosphorothioate can provide the same level of protection obtained by a higher dose. Moreover, some protection was obtained even when vitamin E was administered after irradiation (Srinivasan and Weiss, 1992). But the addition of another nutrient, selenium, to the above combination did not further enhance protection as reported in March 1992 by Srinivasan et al. in the abstract "Radioprotection by combinations of WR-151327, vitamin E, and selenomethionine" at the 40th Annual Meeting of the Radiation Research Society in Salt Lake City, Utah.

The comparative merits of immunomodulators such as synthetic trehalose dicorynomycolate (s-TDCM), detoxified endotoxin (3D-MPL), and interleukin-1 alpha (IL-1 α) were investigated. Although 3D-MPL and IL-1 α are less effective than phosphorothioates as radioprotectors (Kumar et al., 1992), each enhances the survival of irradiated mice when administered in combination with phosphorothioates.

IL-1 α is increased endogenously by the action of endotoxin. Therefore, mechanistic studies of the ability of IL-1 α , 3D-MPL, and s-TDCM to induce endogenous antioxidant systems may reveal their role in radiation protection mechanisms by IL-1 α , 3D-MPL, and s-TDCM.

After IL-1 α administration, we observed an increase in Mn superoxide dismutase (SOD) in liver and a differential effect on MnSOD and CuZnSOD in bone marrow (Kumar et al., 1992). The role of SOD as a radioprotector when it is induced intracellularly may be quite different than when it is given exogenously. Some low molecular weight SOD mimics are radioprotective (Mitchell et al., 1990).

Phosphorothioates cause decrement in the ability of experimental animals to perform motivated tasks (Bogo, 1988) and locomotor activity (Landauer et al., 1992). They also cause nausea and vomiting in humans (Glover et al., 1988). The ra-

dioprotective efficacies and behavioral toxicities of specific phosphorothioates (WR-2721, WR-3689, and WR-151327) were similar when each was administered to mice, intraperitoneally or orally, at a fraction of the lethal dose, LD₁₀.

Norepinephrine and dopamine, which are important neurotransmitters in the central and peripheral nervous systems, can modulate locomotor activity and possibly affect performance of mice. The contents of these neurotransmitters in hypothalamus were tested after treatment with a combination of caffeine and phosphorothioate. We reported earlier that the behavioral decrement caused by phosphorothioate alone was mitigated by simultaneous administration of caffeine (Landauer et al., 1991). Although the contents of norepinephrine and dopamine in the hypothalamus were not affected by phosphorothioate, per se, they did decrease the caffeine-elevated levels of the neurotransmitters to normal (Palazzolo, 1992).

The implications of this novel finding are being explored further by *in vitro* and *in vivo* perfusion. These studies will confirm our selection of proper agents for mitigating behavioral decrement without affecting protection.

Thus, behavioral toxicity seems to be closely associated with protective doses of agents. Therefore, we hypothesized that, while low doses of single agents administered alone provide little or no radioprotection, low doses of single agents administered in combination provide a synergistic radioprotection without interfering with the performance of motivated tasks.

To test this hypothesis, we used four agents: WR-3689 (S-2-(3-methylaminopropylamino)ethyl phosphorothioic acid), the detoxified endotoxin derivative 3D-MPL (Ribi Immunochem, Hamilton, Mont.), and two eicosanoids (misoprostol from Searle, Skokie, Ill.; iloprost from Schering, Berlin, Germany).

A comparison of the radioprotective properties of the three commonly used phosphorothioates (WR-2721, WR-151327, WR-3689) at a low dose of 50 mg/kg revealed no statistically significant difference in protection at 9-Gy irradiation (Kumar et al., 1992). WR-3689 at that dose was not behaviorally toxic (Landauer et al., 1992).

The 1 mg/kg doses of iloprost and misoprostol used in this study are toxic, but preliminary studies with lower nontoxic doses indicate they also provide protection. Studies are in progress to determine the maximum nontoxic doses that provide protection without affecting performance.

The lowest protective dose of 3D-MPL was 8 mg/kg when administered 6 hours before 9-Gy irradiation. In current studies a 0.5-mg/kg dose provided no protection when given 30 minutes before 9-Gy irradiation. That dose caused no behavioral toxicity (Landauer et al., 1992).

The combination of WR-3689 (50 mg/kg) and 3D-MPL (0.5 mg/kg) showed no behavioral toxicity, yet provided an LD_{50/30} at approximately 9.5 Gy compared with 8.14 Gy for controls. Those two agents, combined with 1 mg/kg of each of the eicosanoids iloprost and misoprostol, increased the LD_{50/30} to 12.4 Gy (fig. 1), resulting in a synergistic radioprotection. None of these agents administered alone protected at this radiation dose.

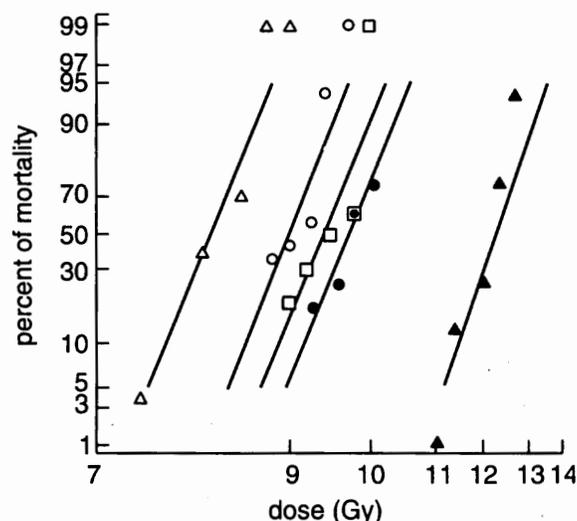


Fig. 1. Probits for protection of irradiated CD2F1 male mice by a combination of four agents. Each drug or combination of drugs was administered as a single intraperitoneal injection 30 minutes before irradiation at a dose rate of 1 Gy/minute (⁶⁰Co, bilateral). (Δ) saline control; (○) WR-3689 (50 mg/kg), DRF 1.10; (□) WR-3689 (50 mg/kg) + 3D-MPL (0.5 mg/kg), DRF 1.16; (●) WR-3689 (50 mg/kg) + 3D-MPL (0.5 mg/kg) + misoprostol (1 mg/kg), DRF 1.19; (▲) WR-3689 (50 mg/kg) + 3D-MPL (0.5 mg/kg) + misoprostol (1 mg/kg) + iloprost (1 mg/kg), DRF 1.52.

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DNA structural organization and its modification by radiation and radioprotectors

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Project 00145

Through this project, we expect to develop a detailed understanding of the radiation sensitivity of local DNA conformational structures and to clarify how radioresistant agents alter DNA structure and confer protection from ionizing radiation. In addition, the project investigates whether other cellular components (e.g., proteins) interact or are modified by exogenous drug administration. Current research has focused exclusively on *in vitro* experiments. In the future we will extend our *in vitro* findings to single-cell systems with attention directed towards understanding and developing methodologies to suppress mutagenesis.

During this past year, a reverse-phase high-performance liquid chromatography (HPLC) methodology was validated for rapid, sensitive, and simultaneous analysis of all stereoisomers of thymidine glycol (Vaishnav et al., 1992). The procedure involved direct injection of the samples on a microbore C-18 reverse-phase column with ultraviolet detection at $\lambda=220$ nm. The lower limits of detection for all thymidine glycol stereoisomers were close to 2.5 pmole and linear up to at least 5,000 pmole. The procedure allowed qualitative as well as quantitative measurements of pH and temperature-dependent interconversions of the isomers underivatized from aqueous solution. Figure 1 is an example of the HPLC product profiles of the reaction mixture from osmium tetroxide-induced oxidation of thymidine after temperature treatments.

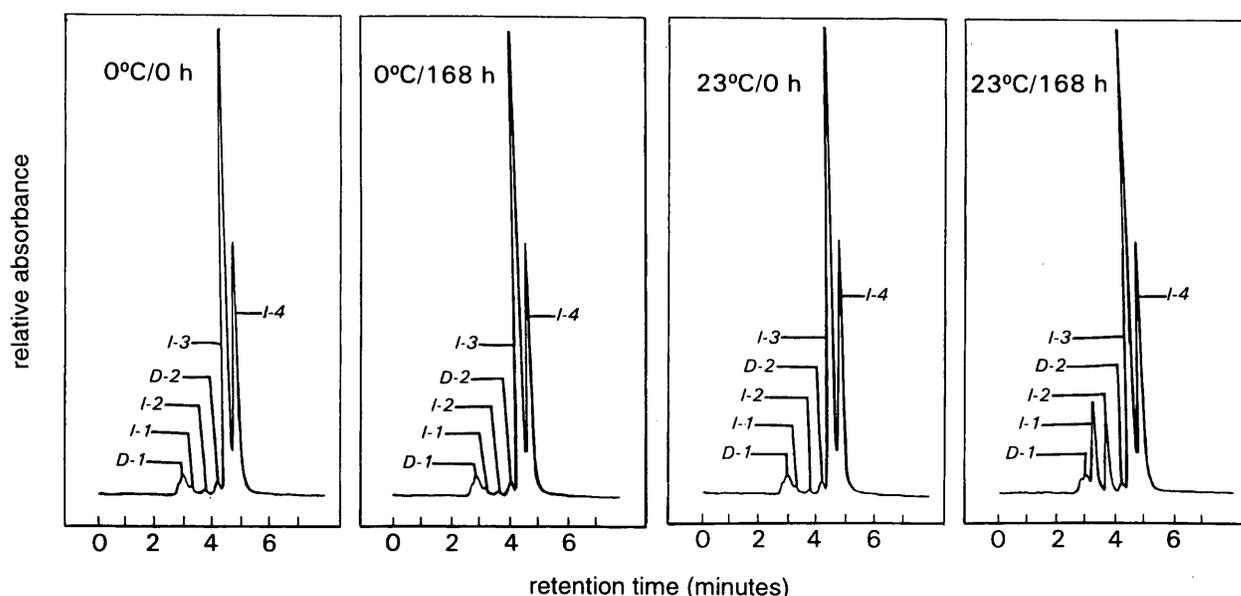
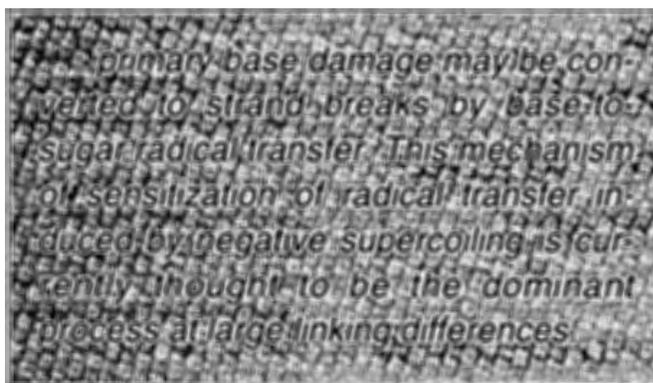


Fig. 1. HPLC product profiles of the reaction mixture from osmium tetroxide-induced oxidation of thymidine after temperature treatments. I-1 to I-4 and D-1 and D-2 refer to thymidine glycol isomers 1 to 4 and dimers 1 and 2, respectively.

Our preliminary study as to whether there are base sequence effects in the radiation sensitivity of DNA has been restricted to single- and double-stranded DNA in the presence and absence of cysteamine (Mao et al., in press). Figure 2(A) illustrates a densitometer tracing of a Maxam-Gilbert gel for the 11-mer duplex (dCACATGTACAC) exposed to gamma (γ) irradiation although the effects of fission neutron irradiation are quite similar. In these experiments, one strand of the duplex was labeled at the 5'-end with [γ^{32} P] adenosine triphosphate (ATP) and employing a T₄ polynucleotide kinase 5'-terminus labeling system. In the presence of 10 mM of cysteamine, a large reduction in strand breaks formation is evident as figure 2(B) illustrates.

It is exceedingly difficult to quantitate these results. However, if we define a protection factor [(PF)=(PF₀)/PF_{cyst.}] as the ratio of areas under the densitometric traces due to damaged fragments in the absence and presence of the radioprotector, then PF=16 for γ irradiation, and PF=2.3 for fission neu-



tron irradiation. This methodology is currently being extended to more complex tertiary DNA structures, namely, hairpins and Holiday structures, which are thought to be important intermediate structures involved in chromosomal alterations.

Supercoiled plasmid pIBI30 (2926 base pairs) was used to investigate whether the topology of closed supercoiled DNA affects its radiation sensitivity. This study addresses whether standard target theory (Fowler, 1964) is applicable to small domain sizes wherein different conformational states can coexist.

Several families of negatively supercoiled topoisomers of the plasmid pIBI30 were ⁶⁰Co irradiated and assayed for the induction of strand scission by agarose gel electrophoresis. Form-I DNA for all topoisomers decreased exponentially as the dose increased. The radiation sensitivity (1/D₃₇) was dependent on the average linking difference (ΔL) associated with a given supercoiled family. The linking difference provides a global measure of

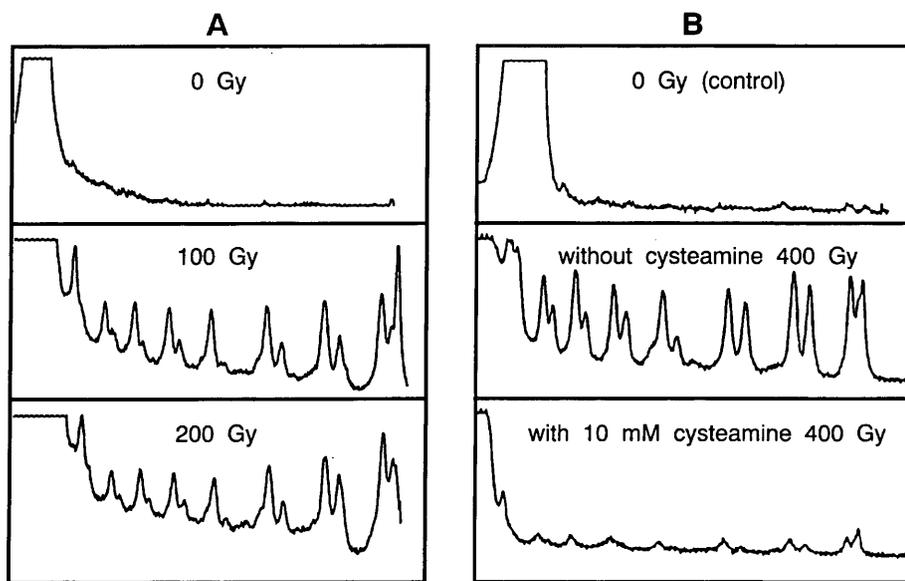


Fig. 2. (A) Densitometer tracings of an electrophoresis gel of the DNA duplex X:Y exposed to γ radiation. (B) Densitometer tracings of the electrophoresis gel of the DNA duplex X:Y exposed to γ radiation with and without cysteamine (10 mM).

system conformational structure. Plasmids having larger linking differences correspond to smaller target volumes. As illustrated in figure 3, for $|\Delta L|$ less than 2.5, the radiation sensitivity of DNA decreased with increasing $|\Delta L|$, a result consistent with target theory (Fowler, 1964), whereas for $|\Delta L|$ greater than 2.5 radiation sensitivity increased with increasing linking difference.

A detailed theoretical explanation of the inadequacies of simple target to small organized conformation states is not yet available although there are at least four competing mechanisms operative. Under our experimental conditions, DNA is primarily attacked by reactive species produced in the aqueous environment and is a more likely pathway for DNA damage than direct ionization. The compact tertiary structure of supercoiled DNA should make it less sensitive to the indirect mode of radiation damage. Similarly, the more negatively supercoiled the DNA, the more likely it is that transient strand separation will occur, thereby reducing the radiation sensitivity of DNA. This protective mechanism follows from the shielded base hypothesis of Ward (1975) and Ward and Kuo (1978). Both mechanisms are consistent with target theory and are inconsistent with our experimental results for $|\Delta L|$ greater than 2.5 (Swenberg et al., in press).

One possible mechanism by which DNA radiation sensitivity increases with increasing linking difference is a change in the energetics of the beta-phosphate elimination pathway (Beesk et al., 1979) that follows hydrogen abstraction from the C4 position of sugar moieties. This suggests that OH radical addition to the double bonds of DNA bases is energetically favored in relaxed DNA over abstraction of hydrogen from ribose. This makes strand scission more competitive with base damage as the elastic energy stored in supercoiled DNA increases. In addition there may be exceptions to the general rule that base damage does not lead to strand scission in duplex DNA as has been found for single-stranded homopolymers poly(U) and poly(dA) (Adinarayana et al., 1988).

The single-stranded characteristics conferred to duplex DNA by negative supercoiling probably increase OH radical addition to bases at the expense of hydrogen abstraction from sugar. Nevertheless, a significant fraction of the primary base damage may be converted to strand breaks by base-to-sugar radical transfer. This mechanism of sensitization of

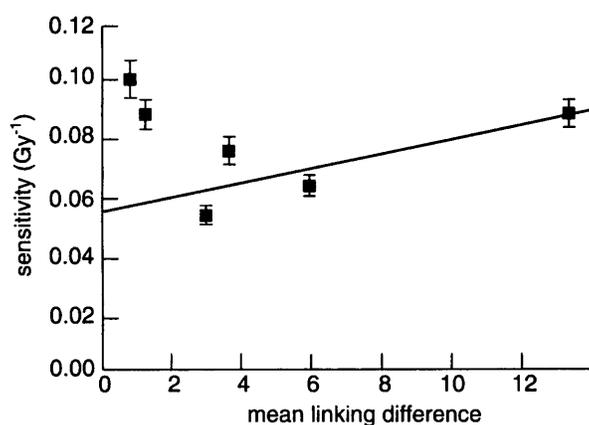


Fig. 3. Sensitivity of topoisomers of pIBI30 to the induction of single-strand scission by ^{60}Co radiation. The data points were obtained by a least square fit of the survival of form-I DNA versus dose.

radical transfer induced by negative supercoiling is currently thought to be the dominant process at large linking differences. Theoretical models are now being developed to quantitatively account for the results shown in figure 3.

Our investigation of thiopolyamine topoisomerase interaction with DNA, previously limited to topoisomerase I interaction with the disulfide WR-33278, has been extended to the monomer WR-1065 and has studied drug concentrations as high as 1.9 mM. Both WR-1065 and its disulfide were found to enhance the activity of calf thymus topoisomerase I relaxation of supercoiled DNA. Results shown in figure 4 clearly indicate this enhanced relationship for both compounds at drug

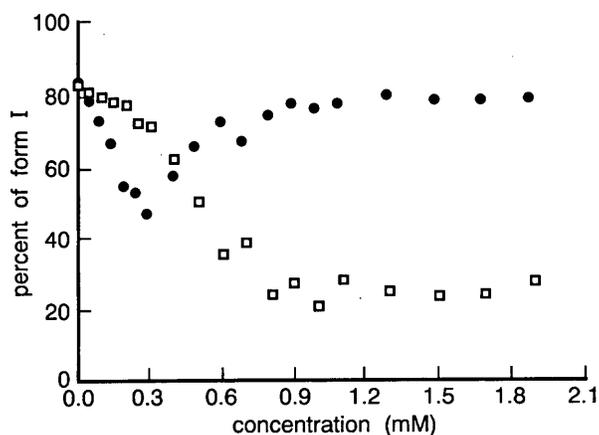


Fig. 4. Percent of supercoiled form-I DNA versus the concentration of WR-33278 (●) and WR-1065 (□).

concentrations less than 300 mM. However, for higher drug concentrations the effect of the disulfide is reduced and saturates at approximately 700 mM whereas the stimulating effects of WR-1065 on topoisomerase I unwinding of superhelical turns is increased with saturation occurring at 800-900 mM. Data from absorption measurements ($\lambda \geq 300$ nm) indicate that the formation of DNA-drug complexes is responsible for the saturation effect of WR-33278 whereas the formation of WR-1065 attributes to the saturation of WR-1065. Our measurements of reduced and oxidized components of each radioprotective compound as a function of time in the reaction buffer, measured by the HPLC/EC (electron capture) detector system, supports this interpretation.

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Effect of radiation on membrane structure and function

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Project 00150

In this project, we seek to understand how cell structure and function are altered by a range of radiation effects that involve or are mediated by cellular membranes.

The research includes investigations of radiation-induced damage to the nuclear envelope, the role of *ras* gene expression in radiation resistance, and alterations in signal transduction pathways after radiation exposure. Our ultimate goals are to provide protocols or agents that inhibit alterations to processes or sites damaged by radiation and to stimulate natural mechanisms in the cell to reverse the damage.

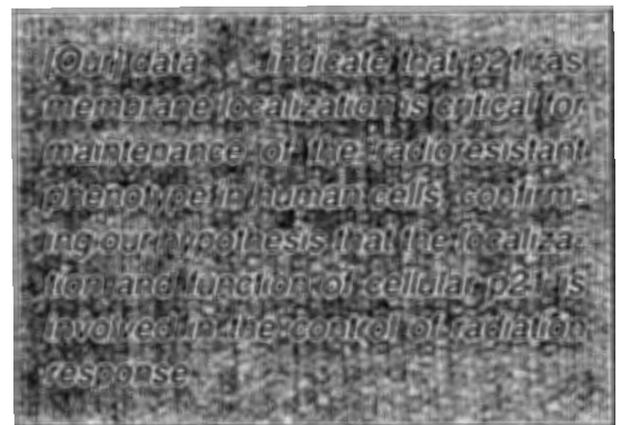
Radiation alters nuclear transport

The nuclear envelope plays a critical role in normal cell homeostasis by regulating the interactions between the genetic information contained in the nucleus and the synthetic and metabolic func-

tions in the cytosol. Clearly, any disruption of the communication between these two compartments could have important consequences for the cell.

In our effort to define the role of the nuclear envelope in radiation injury, we have demonstrated several radiation-induced structural and/or functional alterations of the envelope. These include changes in the kinetics of nuclear protein transport across the nuclear envelope through the nuclear pore, changes in the lipid structure of the nuclear envelope, and changes in the ionic balance between the cytoplasm and nucleoplasm. Although these observations require further characterization, it is becoming evident that the nuclear envelope is an important site of damage in the irradiated cell.

A critical development in the study of radiation effects on nuclear transport has been our establishment of a novel *in vivo* nuclear transport assay that allows us to monitor radiation-induced alterations in transport and to explore the mechanisms involved. This assay uses an *in vitro* transcription/translation system to produce a radiolabeled transport marker protein, nucleoplamin, that contains a nuclear localization signal that targets the molecule to the nucleus. This assay demonstrates most of the same characteristics observed with nuclear transport *in vivo*. That is, proteins without a nuclear localization signal are not transported; transported proteins remain associated with the nucleus; the proteins are not accessible by proteases or detergents; and transport is dependent on adenosine 5'-triphosphate (ATP) (Kalinich and McClain, 1993). Using this assay system, we have demonstrated that radiation stimulates an increase in the kinetics of transport of nuclear-directed proteins.



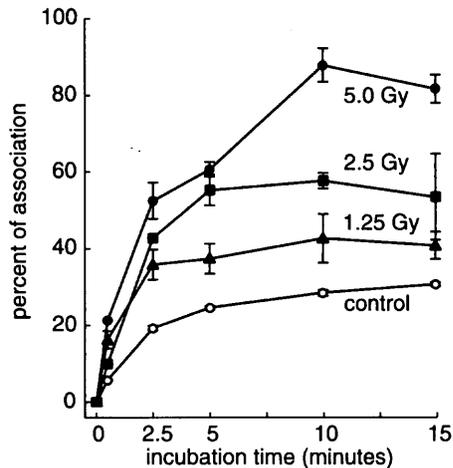


Fig. 1. Effect of radiation on the kinetics of transport of large-T antigen into the nucleus. MOLT-4 lymphocytes were irradiated at the indicated doses with ^{60}Co gamma (γ) radiation and then returned to 37°C for 6 hours. Then nuclei were extracted and purified and the nuclear transport kinetics were determined as described previously (McClain and Kalinich, 1991; Kalinich and McClain, 1993). Percent association is defined as the amount of large-T antigen transported relative to the total amount present during the incubation.

That increase is dependent on radiation dose (fig. 1) and time postirradiation (McClain and Kalinich, 1991).

A limitation of this system has been the presence of rabbit reticulocyte lysate in the translation mixture, which contains natural factors that mediate transport. When the assay is performed, these factors can compensate for deficiencies produced by radiation in the test nuclei, making it more difficult to define mechanisms of radiation damage. In order to circumvent this problem, we have successfully placed the *Xenopus leavis* nucleoplasm gene into *Escherichia coli* (Kalinich and McClain, 1991), which allows us to obtain the purified protein without transport factors such as those found in the reticulocyte lysate. Using an in vitro method that we developed to radiolabel the protein with ^{35}S at high specific activity (Kalinich and McClain, 1992), we have begun to dissect mechanisms by which radiation alters nuclear transport.

Toward that goal we have isolated two factors present in the reticulocyte lysate that are components of the transport process. Factor A is required for the docking of the protein at the nuclear pore, and factor B is involved in the translocation of the protein through the nuclear pore (Moore and Blobel, 1992). Heat shock protein 72 kD and heat shock

protein cognates also appear to be involved (Goldfarb, 1992). The involvement of these stress proteins in nuclear transport may be an important link in the mechanisms of radiation damage since radiation has been shown to induce the production of stress proteins in several systems (Williams et al., 1989). Work is currently under way to determine whether radiation modifies the interaction of such factors to alter nuclear transport.

Radiation alters calcium homeostasis in irradiated nuclei

Because inorganic ions such as calcium appear to play a regulating role in nuclear transport, we have become interested in how radiation might alter calcium metabolism in ways that affect the transport process. The nuclear envelope has recently been shown to partition calcium between the nucleoplasm and cytoplasm (Nicotera et al., 1989). Using fluorescent calcium-binding probes, we have shown that radiation exposure decreases the intranuclear calcium concentration in nuclei from irradiated MOLT-4 cells to a level 80% that of nonirradiated control nuclei. Incubation of nonirradiated nuclei with ATP stimulates a nearly 30% increase in intranuclear calcium concentration; however, irradiated nuclei are virtually insensitive to ATP stimulation. As shown in figure 2, intact MOLT-4 cells demonstrate an analogous insensitivity to ATP stimulation after irradiation (McClain and Kalinich, 1992). Although the pathways that involve calcium are not well understood in either ATP-stimulated nuclei or intact cells, we seek to understand these mechanisms so as to assess their role in the radiation-induced impairment of nuclear function.

Role of membrane damage in radiation-induced apoptosis

Lymphocytes undergo programmed cell death, termed apoptosis, after relatively low doses (1-5 Gy) of ionizing radiation. Even though a variety of morphological and biochemical events have been characterized in radiation-induced apoptosis, the mechanism remains unclear. DNA degradation is the most characteristic marker for apoptosis (Ramakrishnan and Catravas, 1992) and is thought to be mediated by a calcium-dependent nuclear endonuclease, not the direct interaction of radiation-induced free radicals with DNA. Radiation induces

DNA fragmentation by a process that appears to be related to a significant influx of extracellular calcium. The antioxidants trolox, a vitamin E derivative, and dihydrolipoic acid have been shown to inhibit chromosomal DNA degradation in irradiated thymocytes (as reported by Ramakrishnan et al. in May 1992 at the 5th Annual Dinner Meeting of the Oxygen Club of Greater Washington, Bethesda, Md.). In collaboration with Dr. Narayani Ramakrishnan of AFRRI, we have demonstrated that an uptake of calcium does not occur in thymocytes treated with trolox or dihydrolipoic acid. We think that these antioxidant drugs protect DNA by inhibiting radiation-induced membrane damage that leads to the uptake of extracellular calcium.

We have previously demonstrated that EPR (electron paramagnetic resonance) methods can detect radiation-induced damage in the nuclear membrane of nuclei from irradiated MOLT-4 cells (McClain et al., 1990). We will apply EPR methods and a variety of other physical and biochemical approaches to define the mechanism of trolox and dihydrolipoic acid action, concentrating on their effect on membrane structure and function and on related aspects of calcium metabolism in the irradiated cell.

***ras*-Oncogene expression alters radioresistance**

Alterations in *ras* oncogene expression have been associated with increased cellular resistance to

ionizing radiation. We previously demonstrated that *ras* proto-oncogene overexpression in non-tumorigenic cells is correlated with increased radioresistance that is independent of neoplastic transformation (Samid et al., 1991). Semiquantitative analysis of these data indicates that there may be a threshold level of *ras* expression necessary for acquisition of the radioresistant phenotype. Based on these data, we would expect that agents that affect *ras* expression (quantitatively or qualitatively) alter the responses of cells to ionizing radiation.

Quantitative reduction in *ras* proto-oncogene expression following buthionine sulfoximine (BSO) exposure was previously observed by Miller et al. as reported in April 1990 at the 38th Annual Meeting of the Radiation Research Society in New Orleans, La. Recent data show that this decrease in *ras* proto-oncogene expression is correlated with an increase in cellular radiation sensitivity. As has been previously demonstrated, BSO exposure inhibits glutathione (GSH) synthesis, resulting in a decrease in total cellular GSH levels. The BSO concentrations that down-regulate *ras* mRNA reduce GSH levels to <5% of the control level. Therefore, the change in radiation response of BSO-treated cells involves both a quantitative reduction in *ras* and a decrease in GSH content. Further studies are under way to determine the mechanism of this down-regulation and the extent to which GSH versus *ras* expression is important to the radioresistant phenotype in this murine system.

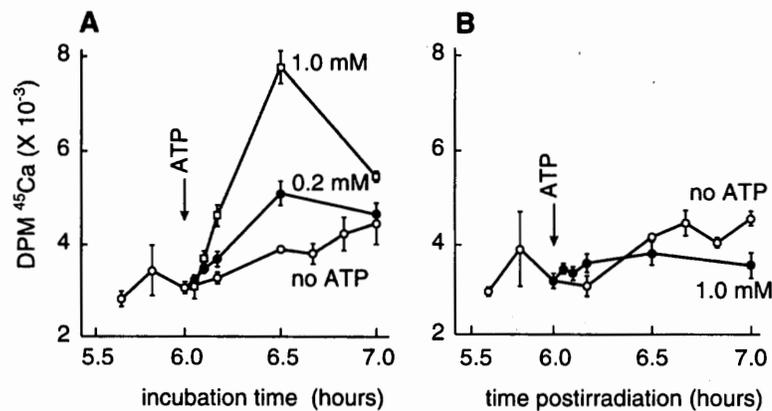


Fig. 2. Effect of radiation on ATP-stimulated calcium uptake by MOLT-4 cells. Cells were irradiated with 5-Gy ⁶⁰Co γ radiation (2×10^7 cells/ml), then incubated 5 hours at 37°C in an atmosphere of air containing 5% CO₂. Then, ⁴⁵Ca (10 μ C/ml) was added to the suspension, and the incubation continued for 1 hour while the association of ⁴⁵Ca was monitored to ensure an equilibration of intracellular calcium stores. ATP (0.2 or 1 mM with nonirradiated cells, 1.0 mM with irradiated cells) was then added (time 0), and the measurement of ⁴⁵Ca association continued in nonirradiated (A) and irradiated (B) cells, both those exposed to ATP and those not exposed to ATP.

As an extension of studies with murine cell models, we have explored the radioresponses of human osteosarcoma (HOS) subclones that differ in their *EJras* expression. Quantitative analysis reveals a close correlation between the amounts of *ras*-encoded mRNA and p21 produced and the degree of cellular radioresistance (fig. 3). Inhibition of

p21^{ras} post-translational processing, via the mevalonate pathway with lovastatin, markedly decreases radioresistance. Protein analysis reveals that lovastatin prevents p21 membrane association in a dose-dependent manner, but does not affect the biosynthesis of p21 (fig. 4). Interestingly, lovastatin does not alter the radioresponses of parental HOS

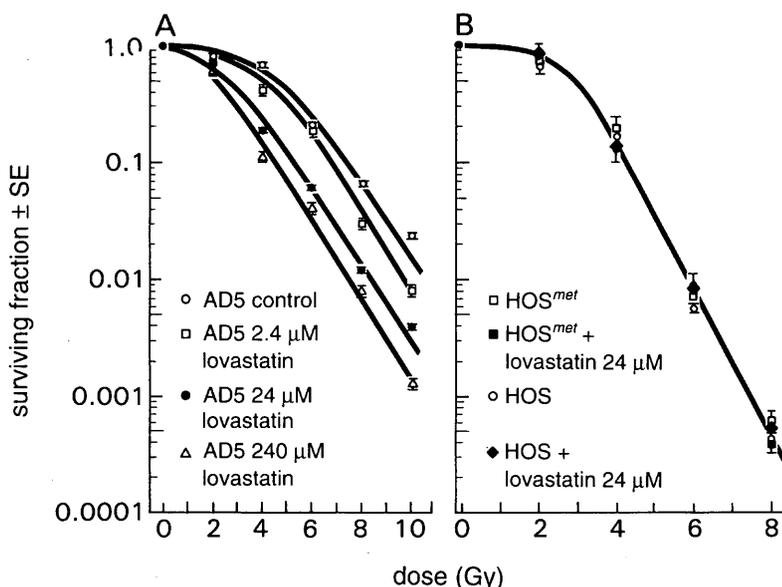


Fig. 3. Radioresistance of HOS-derived cultures. Human osteosarcoma cells with (A) high (AD5) or (B) undetectable (HOS, HOS^{met}) levels of *ras* expression were examined for survival following exposure to ionizing radiation.

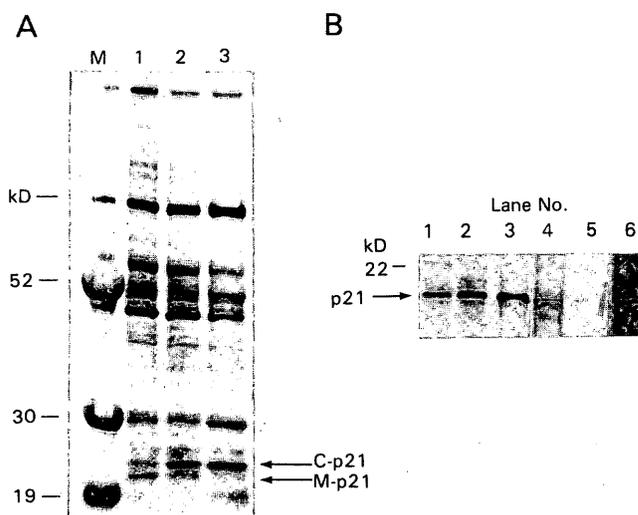


Fig. 4. Effect of lovastatin on p21^{ras} production and membrane localization. (A) AD5 cells were labeled with [³⁵S]-methionine and were treated, concomitantly, for 24 hours with lovastatin (lane 1, untreated; lane 2, 24 μM; lane 3, 240 μM). Following immunoprecipitation with Y13-239 MA b to p21^{ras}, cytosolic p-21 (C-p21) and membrane p-21 (Mp-21) were separated by SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis). (B) AD5 cells were treated for 24 hours with lovastatin (lane 1, untreated control; lane 2, 24 μM lovastatin + 50 μM mevalonate; lane 3, lovastatin 2.4 μM; lane 4, 24 μM lovastatin; lane 5, 240 μM lovastatin; lane 6, 24 μM lovastatin + cholesterol-rich lipids (10 μg/ml). Membrane fractions were immunoprecipitated and separated as above. Molecular weight markers (M) are shown in kD.

cells or HOS cells transfected with an activated met oncogene (fig. 3).

These data, taken together, indicate that p21*ras* membrane localization is critical for maintenance of the radioresistant phenotype in human cells, confirming our hypothesis that the localization and function of cellular p21 is involved in the control of radiation response.

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Radiation Hazards Analysis Program

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Program goals

- Using biophysical techniques, study the fundamental mechanisms of radiation damage in biological systems.
- Develop biophysical models of radiation damage to biological systems.
- Develop extrapolation strategies to permit the use of laboratory data as well as available human data to predict the outcome of exposure to ionizing radiation in novel exposure scenarios.
- Develop means for mitigating or protecting against the late effects of ionizing radiation.
- Develop biological dosimetry techniques to permit the rapid and accurate determination of radiation dose.

Requirement

Current risk assessment and casualty prediction methodologies do not adequately deal with differences between the types of radiation, the radiation energy spectra, or the distribution of dose in the various organ systems of exposed persons. This knowledge is important if we are to establish rational radiation protection standards and predict casualties in a wide range of military and civil scenarios.

Control of the risk of late effects of ionizing radiation is important during scenarios in which personnel enter radiation environments that are not immediately hazardous to their health or their ability to perform their mission, but that result in exposures well beyond current occupational exposure limits. Our efforts in this area will help to develop methodologies to reduce this risk.

Once a person has received a significant exposure, an unequivocal assessment of the magnitude of the exposure is required in order to allow rapid, accurate triage and treatment. Our development of biological dosimetry strategies is designed to meet this requirement.

As a result of Operation Desert Storm, two issues with radiological dimensions were raised. First, because a small number of U.S. service men and women were wounded by depleted uranium munitions, the Army Office of the Surgeon General requested an evaluation of these types of injuries. Second, the Army requested information concerning the use of ionizing radiation to neutralize biological warfare agents. In both cases, ad hoc groups were formed to provide the required information.

Strategy

We will accomplish our goals by using an integrated approach in which biophysical techniques will be applied to available human, animal, and cellular data. Experiments will be conducted as necessary to test hypotheses.

Design study for life-span experiments in mice: Carcinogenesis and biological effects of heavy charged particles

Office of the Director

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Project 04010

This design study proposes to determine how to proceed with life-span studies on the carcinogenic effects of heavy charged particles in rodents. The product of this effort will be a comprehensive report that provides NASA staff prioritized recommendations from recognized experts regarding various experimental approaches that conform to NASA's research requirement.

A pressing need exists for data on carcinogenic effects of heavy charged particles after single or protracted exposures in order to address matters of risk management for personnel in space. Initial slopes of dose-response curves must be defined with the highest priority assigned to murine tumors where the relative incremental risk produced by radiation is thought to be the same as in humans (Fry and Storer, 1987). Data are needed on the carcinogenic risk of heavy charged particles in relation to their charge and velocity so that comparisons can be made with the extensive data base that already

exists for photons and fission-spectrum neutrons. Effects of charged particles will be expressed both in terms of dose and fluence. Owing to the highly complex nature of energy deposition by heavy charged particles, expressing effects in terms of fluence and cross sections may be advantageous, particularly at LET (linear energy transfer) values in excess of 100 keV/ μm .

A great number of uncertainties exist regarding carcinogenic effects of single or protracted doses of heavy charged particles (Ainsworth, 1986). Our ultimate goal is to reduce the uncertainty of prediction of cancer risk in rodent or other experimental systems so estimates of relative biological effectiveness and cross section can be made. This will be accomplished by the collection of a more adequate data base, the use of appropriate analysis methods, and an improved understanding of how to extrapolate animal results to man.

The relevant data collected from in vivo experiments are limited to a comprehensive series of studies on Harderian gland carcinogenesis conducted by Fry et al. (1985) and Alpen et al. (1983). The studies used fission-spectrum neutrons as well as the various heavy charged particles produced by the Lawrence Berkeley Laboratory's BEVALAC, which is a coupling of the heavy-ion linear accelerator (HILAC) and the billion-electron-volt synchrotron (BEVATRON) accelerator.

Harderian tumorigenesis is a superior model system that permits definition of the initial slopes of dose-response curves over a range of low doses (5-60 cGy) of fission neutrons and some HZE (high-charge-and-energy) particles. The model uses pituitary glands transplanted beneath the spleen capsule to provide an excess of various hormones and promote tumor growth or expression. The full promotion paradigm accounts partially for the high sensitivity of the model system, but the initial slope is somewhat affected and the maximum prevalence is greatly influenced by the hormones. The hormonal matter notwithstanding, a critical issue is the extent to which generalizations regarding initial slopes derived from the Harderian system are applicable to dose-response relationships for other tumors in the mouse.

A comprehensive life-span study, supported by histopathology for confirmation of tumor diagnosis, has not been conducted with protons or heavy charged particles. Tumor data from such studies is

critical in the assessment of cancer risks for photons and fission-spectrum neutrons.

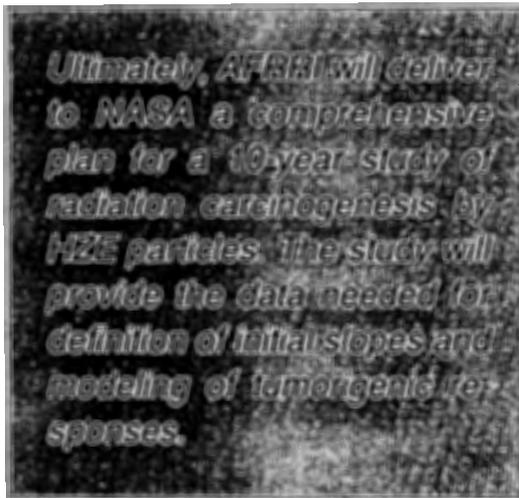
Pilot studies on HZE particles were conducted previously at the Lawrence Berkeley Laboratory and preliminary results have been published (Ainsworth, 1986). Those studies, however, were not designed to evaluate the effects of low doses, and financial support was insufficient for collection and analysis of tumor data. Nevertheless, important new information was obtained on life-span shortening, based on differences in mean survival time for irradiated controls and animals that received single or fractionated doses of heavy charged particles. Data from these pilot studies will be used to estimate the extent of life shortening at low doses and, possibly, expected tumor frequencies. Based on the expectations of tumorigenesis, doses and sample sizes may be tailored accordingly.

An extensive data base on tumors exists for reciprocal hybrid mice used in neutron and gamma ray studies at Argonne National Laboratory (Grahn et al., 1986). The findings of Ainsworth (1986) are consistent with the interpretation that particles ranging from neon to iron are less effective for producing life shortening and, probably, lethal tumors than are fission-spectrum neutrons from the JANUS reactor. These studies used the reciprocal hybrid mouse (CB₆CF₁) that was used in the fission neutron studies at Argonne National Laboratory in order to facilitate comparisons between charged particle and high-LET neutrons.

This effort will provide NASA a comprehensive plan, including various alternatives, whereby the data essential for modeling cancer risks can be obtained. The overall strategy has included (1) organization of the Scientific Advisory Committee (SAC); (2) assessment of the status of information with respect to carcinogenic effects of heavy charged particles; (3) formulation of a tentative experimental design for life-span/carcinogenesis studies; (4) critical assessment of the need for a separate cohort of experimental animals to provide tissues, organs, or other specimens for supplemental

experiments by members of the international scientific community; and (5) assessment of the role of other specific and distinct model systems for carcinogenesis vis-a-vis skin tumors (Burns 1990), mammary tumors (Broerse et al., 1987), myeloid leukemia (Mole, 1986), Harderian gland tumors (Fry et al., 1985) as well as cellular and molecular correlative models.

Ultimately, AFRRRI will deliver to NASA a comprehensive plan for a 10-year study of radiation carcinogenesis by HZE particles. The study will provide the data needed for definition of initial slopes and modeling of tumorigenic responses. These data will be collected from a series of life-span and other shorter term studies. Our intention is to conduct the experiments in such a fashion that sufficient correlative indices will be discovered, and there will be no further need for life-span studies on radiation carcinogenesis in mice with heavy charged particles.



SAC, at its initial meeting on January 10, 1992, at AFRRRI, planned three workshops. The first workshop, conducted in Houston, Texas, on April 16-17, 1992, addressed physics and biophysics problems including radiation transport issues; the relevant fluence, flux, and dose ranges for study; and the fractionation schemes thought to be most equivalent to the actual continuous exposure of astronauts to HZE particles. The relative importance of the risks presented by protons, gamma rays, and HZE particles was discussed at length.

The second workshop, conducted at AFRRRI on September 22-23, 1992, addressed what animal experiments were needed and their design, the most appropriate end points to be scored, and how to extrapolate data from animal experiments to human risk assessments.

The third workshop, to be conducted at AFRRRI on 28-29 April 1993, will address the recent impressive developments in molecular and cellular radiobiology and how such studies can be used in parallel or as part of a life-span study. This workshop will consider the important molecular markers

associated with the phenotypic expression of radio-resistance, or radiosensitivity, and the possible alterations of the phenotypes. It will address whether these markers can be used to evaluate potential radioprotectors. The importance of the transgenic animal model, mainly mouse, that can mimic certain human populations with specific known genetic defects will be discussed.

A fourth workshop to consider new or perhaps inadequately explored topics may be necessary. Specifically, the degrading of advanced cortical activities (e.g., memory, judgment, and concentration) due to heavy particle effects on the mature central nervous system will be an important issue. Plans for the fourth workshop and for a SAC summary session will be made at the third workshop.

The ultimate goal is to provide options to NASA for a 10-year plan for experiments that will satisfactorily resolve all the major questions concerning the carcinogenic risks to humans in space from heavy charged particles.

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Mechanisms of radiation-induced apoptosis and somatic mutations in lymphoid cells

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Radiation-induced cell death can occur either by necrosis or apoptosis. Most mammalian cells undergo necrosis (reproductive cell death) after exposure to low radiation doses. Cells survive the irradiation, but they die after a few cell divisions. This mode of death is characterized at the cellular level by a generalized breakdown of cellular structure and function, followed by cell lysis. In contrast, normal or malignant cells of lymphatic origin undergo apoptosis (interphase death) at clinically relevant doses of 2-5 Gy or less. Apoptotic death is characterized by reduction in cell volume, condensation of the cytoplasm, membrane blebbing, formation of apoptotic bodies, and nonrandom fragmentation of nuclear DNA into oligonucleosomal subunits. Apoptosis is considered an active process in which a programmed sequence of events leads to cell death.

Our earlier studies indicate that thymocytes die by apoptosis following 1-6 Gy of gamma (γ) irradiation (Ramakrishnan and Catravas, 1992a, 1992b). We found that the fragmentation of nuclear DNA into oligonucleosomal subunits, the most characteristic biochemical event in apoptosis, precedes cell

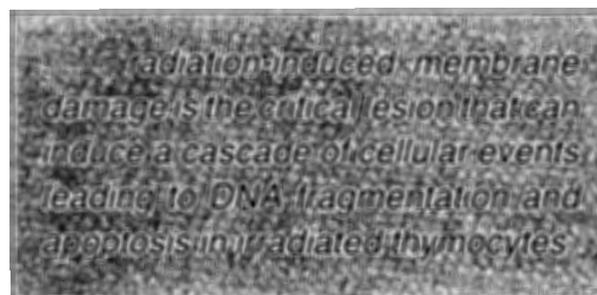
death. The degradation of nuclear DNA during apoptosis appears to be due to activation of a Ca^{2+} -dependent nuclear endonuclease that is constitutively present in an inactive form in thymocyte nuclei (Ramakrishnan and Catravas, 1992a; Vanderbilt et al., 1982). The process by which this enzyme becomes activated is unknown. However, our studies indicate that the induction of DNA fragmentation is related to a concurrent, pronounced flow of Ca^{2+} into the cell.

It is well known that free radicals generated during oxidative stress induced by several chemical and physical agents, including ionizing radiation, react with polyunsaturated fatty acids in membrane and initiate lipid peroxidation (Konings and Oosterloo, 1980). Peroxidative damage to membranes has been shown to disrupt the calcium homeostasis in the cell through oxidation of sulfhydryl groups present in calcium translocases (Pascoe and Reed, 1989).

We used trolox, a specific inhibitor of lipid peroxidation, to investigate the involvement of membrane damage in thymocyte apoptosis induced by low doses of γ radiation. Trolox, a water soluble analog of vitamin E, penetrates biomembranes rapidly and protects mammalian cells from oxidative damage both in vivo (Mickle et al., 1989) and in vitro (Wu et al., 1990).

DNA fragmentation increases in accordance with time and radiation dose. Fragmentation was first detected in irradiated thymocytes at 2 hours after irradiation and had increased to almost plateau level at 8 hours after irradiation. Fragmentation also increased in line with clinically relevant doses (1.5, 3, and 6 Gy) of ionizing radiation.

Postirradiation incubation of thymocytes with 10 mM trolox completely inhibits the fragmentation of nuclear DNA into oligonucleosomal subunits.



The concentration of trolox we used in our studies is not toxic to the cells. After an 8-hour incubation with 10 mM trolox at 37°C under an atmosphere of 5% CO₂, 85±3% (mean ± standard error, n=3) of the thymocytes retains the ability to exclude trypan blue. This compares to a value of 90±3% (n=3) for cells incubated under the same conditions without trolox.

Exposure to trolox only during irradiation does not prevent DNA fragmentation, suggesting it does not work by scavenging free radicals generated during irradiation. Trolox is most effective in inhibiting DNA fragmentation when added to the cells within 30 minutes after radiation exposure. Trolox is known to block the lipid free-radical chain reactions that propagate after irradiation.

Trolox need not be present continuously to exert its effects. Incubation of the irradiated cell suspension with trolox for 2 hours is sufficient to prevent DNA fragmentation measured at 24 hours. This suggests that irradiated cells cannot initiate DNA fragmentation once the free-radical chain reaction is broken by trolox. Trolox completely blocks the influx of Ca²⁺ (in collaboration with David E. McClain, Ph.D., AFRRRI) in irradiated thymocytes. We obtained similar results with dihydrolipoic acid, another lipophilic membrane-protecting antioxidant. The study supports the hypothesis that radiation-induced membrane damage is the critical lesion that can induce a cascade of cellular events leading to DNA fragmentation and apoptosis in irradiated thymocytes.

Apoptotic cell death occurs under a variety of physiological conditions, including embryogenesis, metamorphosis, cytotoxic T-cell-mediated killing of target cells, and the death of autoreactive thymocytes. Hydrogen peroxide generated *in vivo* has recently been identified as a direct inducer of apoptotic cell death in murine blastocysts (Parchment, 1991). *In situ*, thymocytes are constantly exposed to reactive oxygen radicals during inflammatory immune response (Hendricks and Hendrick, 1988).

We used a free-radical-generating model system to investigate whether oxidative stress induces apoptosis in thymocytes. Thymocytes were exposed to oxygen free radicals by treating them with 0.5-10 μM H₂O₂ for 10 minutes in phosphate-buffered saline supplemented with 0.1 mM ferrous

sulfate. Cells were resuspended in RPMI 1640 medium with 10% serum and incubated at 37°C under 5% CO₂ in air.

Electron microscopic studies (in collaboration with Yuan-Hsu Kang, Ph.D., Naval Medical Research Institute) reveal morphological changes characteristic of apoptosis in H₂O₂-treated cells. The cytoplasm and nuclear chromatin become condensed (fig. 1, A-D). The chromatin forms dense crescent-shaped aggregates that line the nuclear membrane (A and B) and are unlike the flocculation of chromatin exhibited during necrosis. Invaginations in the nuclear membrane like those that occur in A and B will ultimately break the nucleus into smaller pieces as seen in B and D. The plasma membrane becomes convoluted (B), an event preceding the formation of apoptotic bodies. These morphological changes are accompanied by internucleosomal fragmentation of nuclear DNA.

We have developed a fluorometric method to quantitate the DNA fragmentation during apoptosis (Ramakrishnan and Catravas, 1992a). DNA fragmentation is negligible immediately after H₂O₂ exposure (8% at 0 hour). DNA fragmentation increases with postexposure incubation time (42% at 8 hours). DNA fragmentation also increases with higher concentrations of H₂O₂ (0.5-10 μM). DNA fragmentation is inhibited in H₂O₂-treated thymocytes following a 2-hour treatment with trolox (10 mM). Treatment of thymocytes with trolox prior to H₂O₂ exposure was also found to protect the thymocytes from DNA fragmentation. However, trolox administered concurrently with H₂O₂ does not protect against DNA fragmentation. The results indicate that oxidative stress induces apoptosis in thymocytes, and this induction can be prevented by trolox.

Somatic mutations induced by radiation and chemicals are known to play an important role in the etiology of cancer. We have developed a mouse model to study ionizing-radiation-induced somatic mutations *in vivo* at the hypoxanthine guanine phosphoribosyl transferase gene in T-lymphocytes (Ramakrishnan et al., 1992c).

The optimum conditions for T-cell colony formation were investigated. They include mitogen treatment, concentration of growth factor, and the number of irradiated L5178Y mouse lymphoma feeder cells. Thioguanine was used for the selection

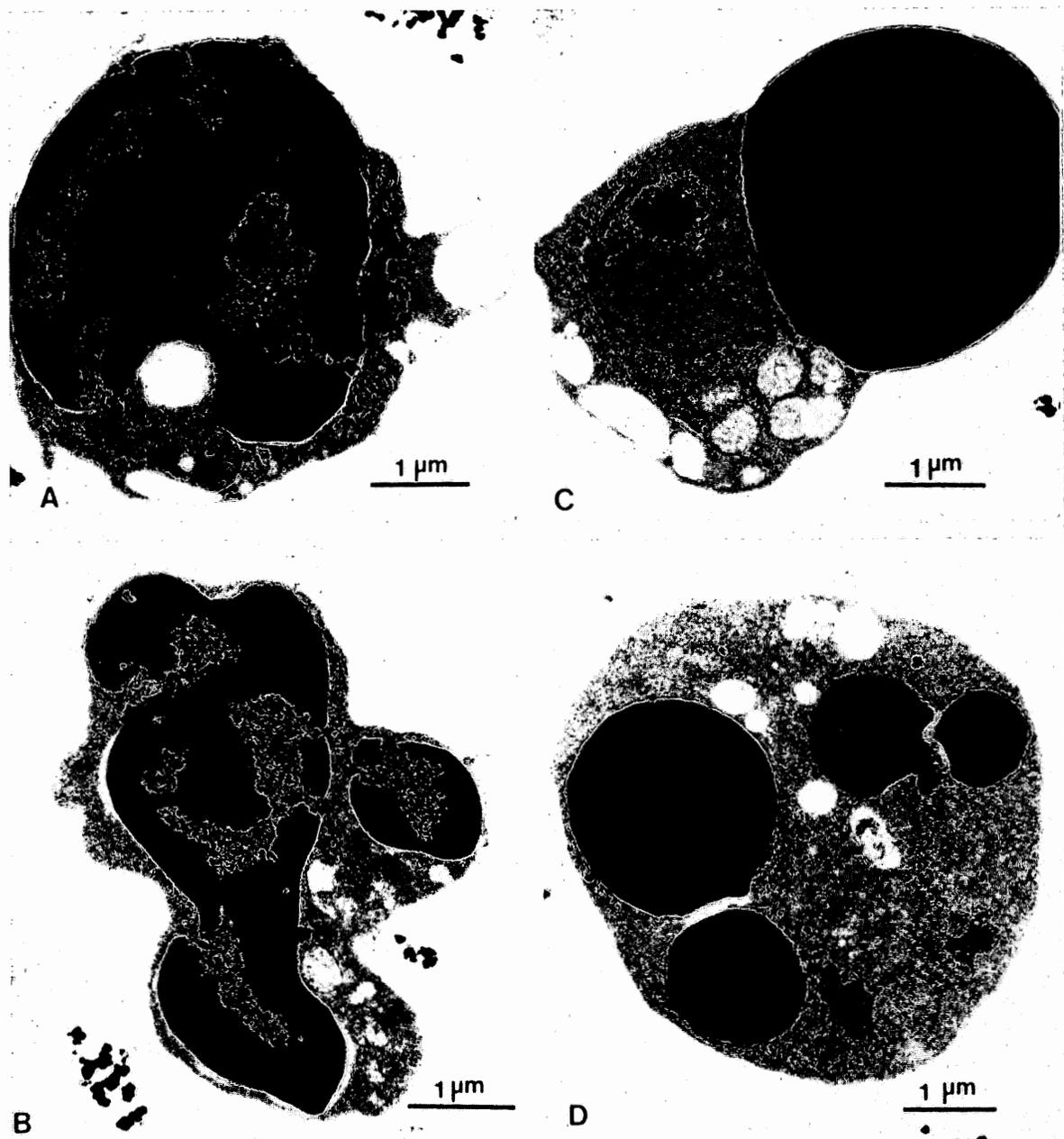


Fig. 1. Morphological features of apoptosis induced in thymocytes by $10 \mu\text{M H}_2\text{O}_2$. Thymocytes exposed to $10 \mu\text{M H}_2\text{O}_2$ for 10 minutes were resuspended in fresh medium and incubated for 5 hours at 37°C under an atmosphere of $5\% \text{CO}_2$ in air. The cells were fixed and dehydrated in a series of graded ethanol solutions and embedded in Epon. Pale gold ultrathin sections prepared with a diamond knife were stained with uranyl acetate and lead citrate. Cells were examined in a JEOL 100 CX II transmission electron microscope.

of mutants. Proliferation of cells was detected by ^3H -thymidine incorporation and liquid scintillation counting. Cloning efficiencies in the selection and nonselection plates were calculated by the Poisson distribution method. The frequency of mutation was obtained from the ratio of cloning efficiency of selection plates to the cloning efficiency of non-selection plates.

Cloning efficiency of lymphocytes is maximum in medium (RPMI 1640 supplemented with 25 mM HEPES buffer, 2 mM glutamine, $55 \mu\text{M}$ 2-mercaptoethanol, antibiotics, and 10% heat-inactivated serum) containing either 5 U/ml interleukin-2 or 10% conditioned medium. There is no colony growth in the absence of irradiated feeder cells. Maximum cloning efficiency is obtained with

1x10⁵ feeder cells per well. Lymphocyte proliferation is maximum after a 48-hour incubation with 2.5 µg/ml concanavalin A in the medium.

We thank William W. Wolfe (AFRRI) for his excellent technical assistance.

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Radiation physics studies

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Project 04610

The overall goal of this project is to determine the sensitivity of microorganisms, toxins, and various chemical agents to the destructive effects of radiation, heat, and pressure produced by a nuclear explosion. Our multidisciplinary approach involves members of AFRRRI's Radiation Biophysics, Radiation Biochemistry, and Experimental Hematology Departments.

On Sept. 18, 1992, an experimental bio-cassette containing bacterial spores was exposed to high doses of neutron radiation at the extremely high dose rates generated by an underground nuclear detonation at the Nevada Test Site. The experimental array consisted of spores of *Bacillus subtilis* and *Bacillus pumilus* and of dosimetry devices contained in an iron cassette. The spores had been impregnated on filter paper strips (3.8 x 0.6 x 0.01 mm) and packaged as bundles of 24 spore strips tightly wrapped in cellophane. The spore strip bundles were loaded into acrylic cassettes that were

placed in Teflon capsules along with the passive dosimetry packages. The dosimetry packages consisted of radiochromic film, alanine pellets, sulfur and niobium activation foils, and neutron sensitive diodes. The iron cassette included a heat shield, iron attenuators, six spore/dosimetry packages, and a temperature chart recorder. The spore/dosimetry packages in the iron cassette were exposed to the direct beam of radiation generated from the underground detonation of a nuclear device. Iron filters were incorporated into the design of the cassette to produce a range of neutron doses. Viable spores were isolated from irradiated and control spore specimens and were counted by standard dilution and plating methods. These data were used to generate spore kill curves (fig. 1).

Previous studies on the radiosensitivity of bacterial spores have primarily used gamma (γ) rays to sterilize medical instruments or food products. Literature searches have revealed no previous studies on the effects of neutron radiation on bacterial spores. AFRRRI's experiments were the first tests of spore killing by neutrons and the first experiments to examine the effects of radiation produced by an underground nuclear test on microorganisms.

For comparison with underground tests, bacterial spores were exposed to γ rays in the AFRRRI ^{60}Co facility and to neutrons generated by the AFRRRI TRIGA reactor. Kill curves were generated by plotting the surviving spore fraction versus the radiation dose. The kill curves showed that the AFRRRI nuclear reactor is an excellent simulator for underground neutron irradiation of biological specimens. The data showed no evidence of a dependence on neutron energy or neutron dose rate (fig. 1).

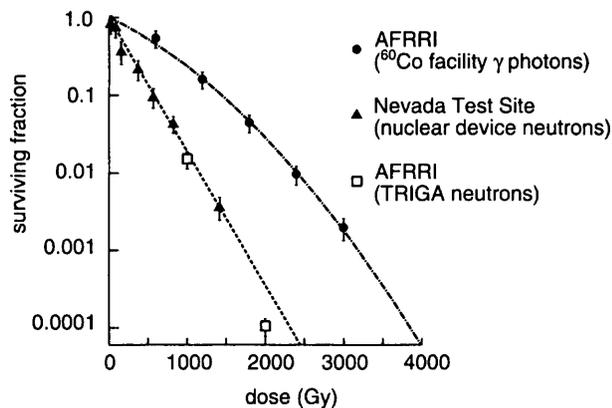


Fig. 1. Spore inactivation by radiation.

To extend this work, experiments will be conducted to systematically answer questions concern-

ing the sensitivity of other microorganisms, toxins, and chemical warfare agents to radiation.

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Life-shortening effects of proton irradiation with and without radioprotection

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The space radiation environment consists of several types of energetically charged particles and include electrons, protons, helium, and heavier nuclei. Outside of radiotherapy (Goitein and Suit, 1985; Lawrence et al., 1966; Tobias and Todd, 1966), humans rarely encounter these charged particles; therefore, little is known about their acute or long-term biological effects.

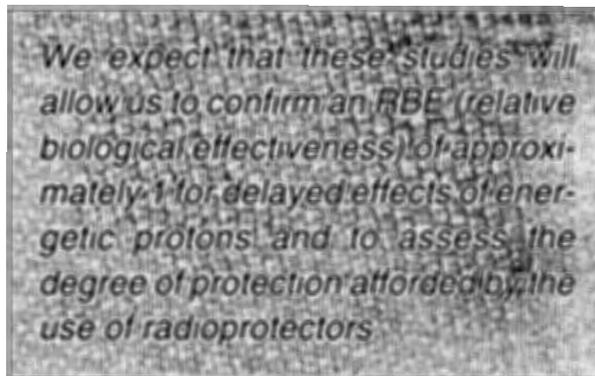
This study focuses on protons, which are the single largest contributor to the total particle fluence (NCRP, 1989) and are considered one of the most hazardous forms of space radiation. The effects of other forms (e.g., electrons, energetic helium, and other heavier nuclei) have been or are being addressed by other studies (Leith et al. 1983; Ainsworth, 1986).

Based on microdosimetry arguments (ICRU, 1970, 1983), it is likely that the effects of high-energy protons will not be significantly different from those of other low-LET (linear energy transfer) radiations such as ^{60}Co gamma (γ) rays. However, this has not been demonstrated for end points relevant to manned missions in space.

We will test the "null" hypothesis; that is, because high-energy protons are low-LET radiation, no differences in long-term biological effects will be observed between protons and other low-LET radiations. Hence, much of the radioprotection data for ^{60}Co γ rays and other forms of low-LET radiation can be applied directly to energetic protons. This study will include single and fractionated doses of proton irradiation as well as the administration of the radioprotector WR-2721 after irradiation.

We expect that these studies will allow us to confirm an RBE (relative biological effectiveness) of approximately 1 for delayed effects of energetic protons and to assess the degree of protection afforded by the use of radioprotectors. Because long-term protracted radiation experiments are not feasible with protons, knowledge gained from protracted, low-LET photon irradiation experiments will be extrapolated to proton irradiation with and without radioprotection.

Our studies will define the biological effects of the space radiation environment as well as the need for and expected results of chemical radioprotection in such an environment. In addition, the life-shortening controls without WR-2721 will constitute a definitive data set that can be considered against other data sets for photons, neutrons, and eventually heavy particles.



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Iron beam fragmentation

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Project 00179

The purpose of this project, which is a joint venture involving AFRRRI and several academic institutions, is to determine how iron beam (^{56}Fe , 600 MeV/amu) fragmentation influences biological effects in mice. Our approach is to determine the relative biological effectiveness of Fe beam fragmentation (FeFRAG) for cell killing, neoplastic transformation, life shortening, Harderian tumorigenesis, and chromosomal aberrations in lens epithelial cells and cataracts.

The mice were irradiated at Lawrence Berkeley Laboratory and transferred to AFRRRI in the fall of 1989 where they have been housed and examined by a number of investigators. The team approach provides maximum information from these valu-

able animals and ensures an efficient and integrated analysis of cancer risk from this form of cosmic radiation.

During 1992, life-shortening data were collected on mice that had received Fe particles in single or fractionated doses of up to 5.1 Gy. The data indicate that fragmentation of the Fe beam, when passed through polyethylene shielding, significantly protects mice receiving a single dose of Fe particle irradiation, but not mice receiving fractionated doses (table 1). Further, the effects of dose fractionation, with and without shielding, were observed (table 2). Without shielding, fractionation did not significantly increase the life-shortening effect of Fe particle irradiation. With shielding, it did.

The effects of FeFRAG are obviously complex and difficult to predict. However, with further analysis, we expect the results of this pilot experiment to demonstrate the relationships between particle velocities and charges and between life shortening and carcinogenesis. That information will assist materially in the development of predictive models for charged particle risk assessment in complex radiation fields.

Table 1. Effect of polyethylene shielding on life shortening in Fe-irradiated mice.

Radiation	Shielding	Dose	Survival time (mean \pm SE)	p
Fe single	0 cm	3.6 Gy	621 \pm 28 days	
Fe single	5 cm	3.6 Gy	739 \pm 21 days	0.0036
Fe 6 fractions	0 cm	5.04 Gy	631 \pm 24 days	
Fe 6 fractions	5 cm	5.04 Gy	597 \pm 23 days	0.337

Table 2. Effect of dose fractionation on life shortening in Fe-irradiated mice.

Radiation	Shielding	Dose	Survival time (mean \pm SE)	p
Fe single	0 cm	3.6 Gy	621 \pm 28 days	
Fe 6 fractions	0 cm	3.54 Gy	608 \pm 24 days	0.392
Fe single	5 cm	5.1 Gy	712 \pm 19 days	
Fe 6 fractions	5 cm	5.04 Gy	597 \pm 23 days	0.0006

DNA damage

Radiation quality and radioprotector effects

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Project 04640

The study of damage and repair to DNA, the genetic material of cells, contributes to the establishment of radiation risk estimates based on fundamental radiobiology concepts (Sinclair and Fry, 1987).

Our goals are to (1) develop an assay for clustered lesions in DNA, (2) automate the scoring of chromosome damage, and (3) examine the mechanism(s) of aminothiols-induced radioprotection of acute (cell killing) versus late effects (mutation and cell transformation) injury following exposure to low- and high-linear-energy-transfer (LET) radiation sources.

Chemical characterization of lethal DNA lesion

DNA clustered lesions or locally, multiply damaged sites (Ward et al., 1985) are composed of base and strand break damage. Previous efforts have concentrated on the measurement of base damage (Fuciarelli et al., 1990; Blakely et al., 1990) using a calf thymus DNA model system. In fiscal year 1992, we initiated the use of a plasmid DNA model system to chemically characterize and investigate the radiobiologic importance of critical clustered lesions in DNA.

In collaboration with Drs. Michael A. Xapsos and Allison A. Stankus, we set up an alternative to photographic detection methodology for quantifying plasmid DNA topological forms resolved in agarose gels. Figure 1 illustrates the system components. The UV transilluminator permits UV excitation of plasmid DNA stained with ethidium bromide. The band pass filter allows selective detection of ethidium bromide-DNA emission (580-630 nm). The video camera is coupled to a personal computer (PC) image analysis system equipped with a video printer.

This fluorescent detection system, compared with conventional photographic systems, affords a remarkable linear dynamic range for DNA detection. It provides a convenient, rapid, and sensitive system for determination of plasmid strand breaks using the agarose gel electrophoresis assay.

Studies using the plasmid model system are investigating the effect of various radical scavengers on the modification of cobalt gamma (γ) rays and fission-neutron-induced DNA damage. The results from these studies will be used in an analysis of the spatial distribution of energy deposition events for low- versus high-LET radiation.

Biodosimetry: Chromosome aberrations

At present, the major cytogenetic laboratories involved in quantifying radiation exposures score chromosome aberrations in conventional metaphase chromosome spreads, micronuclei in cytochalasin-B blocked binucleated cells, and chromosome aberrations in interphase cells that have been fused with mitotic cells to permit examination

of chromatin-like damage, otherwise known as the premature-chromosome-condensed assay (Johnson and Rao, 1970; Iliakis et al., 1992). This latter approach has a number of significant advantages. For example, this assay can be performed in 3 hours in contrast to other assays that typically require 2 to 3 days for the cells to enter mitosis (conventional metaphase spread chromosome aberration assay) and attempt cell division (cytochalasin-B blocked micronuclei assay). When coupled with the chromosome-painting technique, which can involve DNA probes for human centromere sequences (Meyne et al., 1989) as well as specific human chromosomes (Collins et al., 1991), this technique permits ease of scoring for dicentrics, acentric fragments, rings, and translocation aberrations (Lucas et al., 1992).

While these DNA probes are commercially available, the quantities required in our studies generally make them too costly. Therefore, to support our development of automated image analysis methodology for these biodosimetry applications (Cremer et al., 1992), we set up our laboratory for large-scale plasmid isolation and DNA probe synthesis for in situ DNA hybridization studies.

In our initial studies, we have used a plasmid, p82H, which contains a 2.4-kilobase (kb) insert of the consensus sequence for the human centromere region (Mitchell et al., 1985). The 2.4-kb p82H insert has been previously characterized as containing 14 repeats of a 172-base repeated sequence. Using conventional molecular biology methodology, the plasmid was transfected in a bacteria host cell line, isolated by cesium chloride gradient centrifugation, and used as a substrate in a polymerase

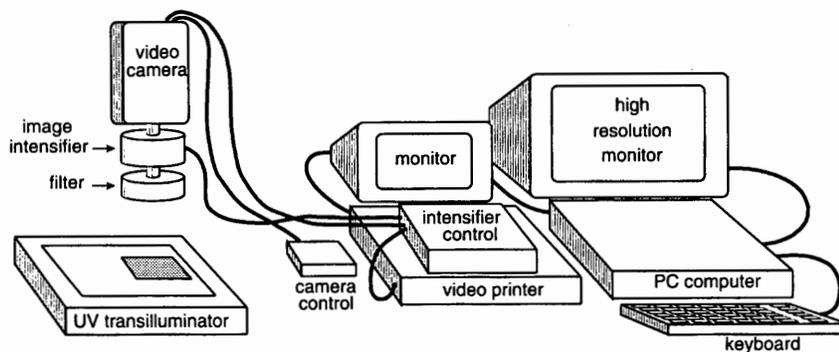


Fig. 1. Schematic for a video camera coupled to a PC image analysis system for quantifying plasmid strand breaks assayed by agarose gel electrophoresis.

chain reaction (PCR). The sequences of the oligonucleotide primers (17 nucleotides) are as follows.

P1: 5'-CCAGACAGAAGCATTCTCA-3'

P2: 5'-GTGTGTTTCAAACCATGCT-3'

They are representative of sequences 39 to 58 (P1) and 137 to 118 (P2) relative to the 172 base pair (bp) alphoid consensus sequence.

The products from the PCR-based synthesis were analyzed by agarose gel electrophoresis (fig. 2). As expected, the synthesis produced a family of products of varying lengths representing the spacing distance between the primer sequences found in the 14 repeats that compose the 2.4-kb insert. These results are consistent with the findings by Weier and colleagues, who used a similar approach for the PCR-based synthesis of murine centromere probe (Weier et al., 1991). Additional efforts are under

way to attach fluorescent labels to these probes so that they may be viewed with a fluorescent microscope following in situ hybridization to human chromosomes.

In collaboration with Dr. E.A. Blakely, experiments examining the effect of radiation quality on the induction of micronuclei in V79 cells were also performed. Cells were irradiated at the BEVALAC (Lawrence Berkeley Laboratory, Berkeley, Calif.) and were scored for micronuclei yield and cell division delay at AFRRRI. Similar experiments were performed at AFRRRI with fission neutrons. The analysis of these results are under way.

Radioprotection: Acute versus late effects

AFRRRI's fission neutron source provides a convenient high-LET radiation source. The recent modification of the reactor to permit the use of an

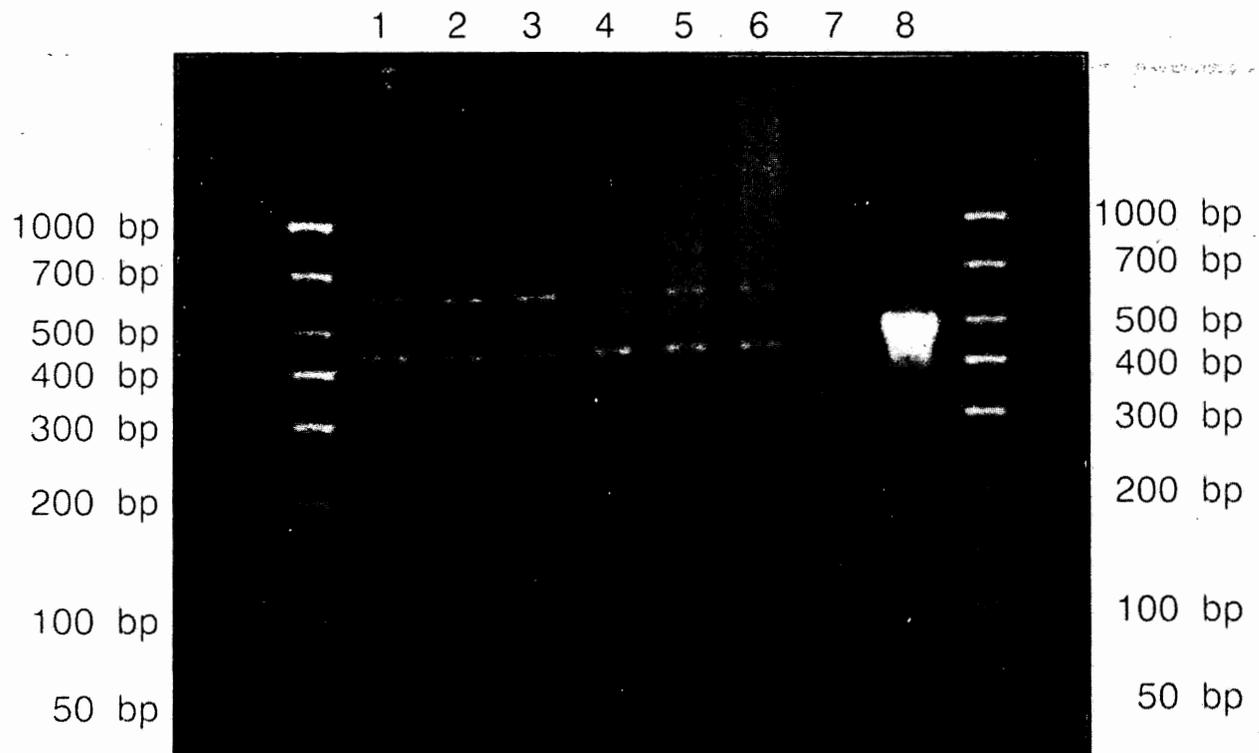


Fig. 2. PCR-based synthesis of human centromeric probe assayed by agarose gel electrophoresis. The two extreme outside lanes were not used. The next set of outside lanes contained a DNA ladder representing 50, 100, 200, 300, 400, 500, 700, and 1000 bp. Lanes 1 to 6 contained aliquots of the PCR product using designated amounts of p82H substrate: (1) 0.1 ng, (2) 1 ng, (3) 10 ng, (4) 0.5 ng, (5) 1.0 ng, (6) 10 ng. Lane 7 was a negative control with no DNA substrate. Lane 8 was a positive control PCR using a 500-bp DNA fragment marker.

extractor tube to move samples in and out of the lead cave setup in the reactor exposure room has made this source more useful in radiobiology studies.

We performed a characterization of the effect of neutron radiation on cell killing using clonogenic survival dose response curves. A characterization of the effect of fission neutron radiation on cell killing was performed for both Chinese hamster V79 cells and human CGL1 hybrid cells. Figure 3 illustrates the neutron relative biological effectiveness for CGL1 cell killing relative to cobalt γ rays. The studies with CGL1 cells support experiments involving a collaboration with Dr. J.L. Redpath and address the inverse-dose-rate effect for neutron-induced cell transformation (Redpath et al., 1991).

In support of the central theme of this part of our study, we examined the effect of aminothiols treatment on modification of fission neutron-induced cell transformation in C3H10T $\frac{1}{2}$ cells. A moderate WR-1065 dosage (1 mM, 30 minutes before and after irradiation), which caused negligible effects on radiation-induced cell killing, caused a 3.3 dose modifying factor protective effect on fission neutron-induced cell transformation as

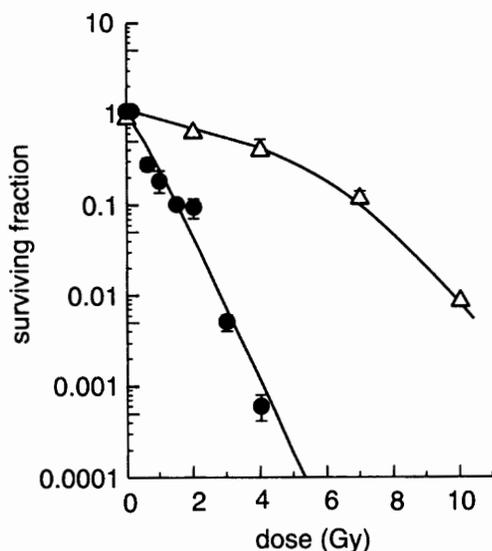


Fig. 3. Clonal survival versus radiation dose response curves. Human CGL1 hybrid cells exposed to cesium 137 γ rays (Δ) and AFRRRI fission neutrons (\bullet). The cells exposed to γ rays were allowed 6 hours of postirradiation incubation before being replated for survival measurements (Redpath et al., 1987). The cells exposed to neutrons were replated for survival measurements immediately after radiation exposure.

shown in figure 4 (Balcer-Kubiczek et al., 1993). These results are consistent with the mutation studies by Grdina and colleagues (Grdina et al., 1988) and support the potential useful benefits of aminothiols therapy for radioprotection against late effect end points (mutation, cancer, etc.).

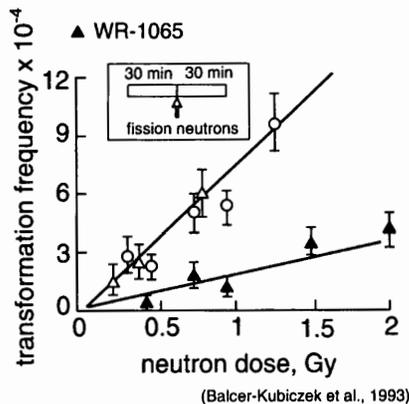


Fig. 4. Effect of WR-1065 on cell transformation by fission neutrons. The neutron-only dose-response curves represent a fit to data for exponentially growing C3H/10T $\frac{1}{2}$ cells exposed to JANUS neutrons (Δ) at Argonne National Laboratory and to density-inhibited C3H/10T $\frac{1}{2}$ cells exposed to TRIGA neutrons (\circ) at AFRRRI. Cells were treated with WR-1065 at 1 mM 30 minutes before and 30 minutes after exposure to TRIGA neutrons (\blacktriangle).

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Utah, March 1992

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Role of oxygen- and nitrogen-centered free radicals in pathophysiological conditions

EPR and spin trapping techniques

Radiation Biophysics Department

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Project 04630

This project measures the mechanisms of free-radical damage in biological systems. It also investigates the protective mechanisms against free-radical damage. A free radical is a reactive molecule containing an unpaired electron. Radiation damage to biological systems is mediated by free-radical pathways. Electron paramagnetic resonance (EPR) spectroscopy is the primary technique used to study free radicals and their reactions. The main focus of this project is the response to ionizing

radiation of oxygen- and nitrogen-centered radical pathways in the intestine. We have chosen the gut as our principal model system since (1) it is one of the primary targets of radiation injury, (2) it has an extensive vascular and nervous system, and (3) it has two layers of smooth muscle. The information obtained can be applied to radiation-induced central nervous system, vascular system, and muscular disorders.

The effect of ionizing radiation on oxygen- and nitrogen-centered radicals in biological systems and on the delicate balance between biochemical pathways may play a key role in radiation-induced mutagenesis, carcinogenesis, vascular dysfunction, muscular disorders, and behavioral changes.

This project investigates the macromolecular events associated with oxygen-derived and NO[•]/NO-related free radicals (second messengers) in response to ionizing radiation and is vital to the development of potential pharmacological agents (radioprotectors) to ameliorate the early or late effects of radiation injury. We hope to elucidate the biochemical balance between the oxygen and nitrogen species in order to provide a mechanistic explanation of how oxygen- and nitrogen-centered free radicals are produced, how their breakdown is controlled, and how these metabolic processes are perturbed by ionizing radiation.

Free-radical pathways have been thought to play a major role in vascular disorders in various organs (gut, lung, heart, kidney, brain, liver) and have been linked to toxicity of various xenobiotic agents in these organs. Generally, attention has focused on free-radical-induced pathological conditions involving oxygen-related species. These include superoxide (O₂^{•-}), hydrogen peroxide (H₂O₂), and hydroxyl radicals (•OH).

However, in recent years, a new important class of biologically generated free radicals has emerged. These are the nitrogen-centered radicals nitric oxide (NO[•]) and nitrogen dioxide (NO₂). NO[•] is a second

messenger and its production is known to occur in several types of cells. It is known to be directly involved in the mechanisms controlling vascular vessel tone (endothelial-derived relaxing factor, EDRF) and in the cytotoxic mechanisms originating from macrophages. Furthermore, O_2^- reacts rapidly with NO^* and is known to be the major mode of EDRF inactivation. Therefore, for a complete understanding of free-radical involvement in pathological conditions, we should exclude none of these species but, instead, focus simultaneously on the roles of and the relationship between species such as O_2^- and NO^* as depicted in figure 1.

Although the EPR and spin trapping techniques are well established in biological studies of oxygen-centered radicals, their application to the study of the role of NO^* is just emerging.

In the EPR facility at AFRRI, several studies focus on production mechanisms and biological effects of radicals such as O_2^- and NO^* . Studies of primary interest investigate the effects of ionizing and light radiation on the delicate balance between these radicals, the mechanisms that protect against their deleterious effects, and the biochemical pathways. Under investigation are, at the cellular level, endothelial, macrophage, and thymocyte cells and, at the tissue organ level, arteries (aortic, caudal), the hippocampus, and the gut. We have used a combination of biophysical, spectroscopic, and physiological approaches to determine the biochemical markers of cellular end points caused by the generation of free radicals (particularly, oxygen and nitrogen radicals, which can act on genetic material as well as on living cells and tissue).

Since radiation injury is caused by free radicals produced during oxidative stress, protection against this stress is important. Using the EPR kinetic assay for superoxide dismutase (SOD) developed during 1991, we evaluated manganese-desferral complexes reported to be SOD mimics. This evaluation established a sensitive kinetic EPR method that can be used as a model and applied to other known antioxidants and radioprotectors.

The method consists of reacting the antioxidant and the spin trap 5,5-dimethylpyrroline-1-oxide (DMPO) with superoxide anion radicals in a competitive manner. CuZnSOD, MnSOD, and FeSOD enzymes function by decomposing superoxide anion radicals and, thus, preventing oxidative stress in cells. SOD enzymes cannot cross the cell mem-

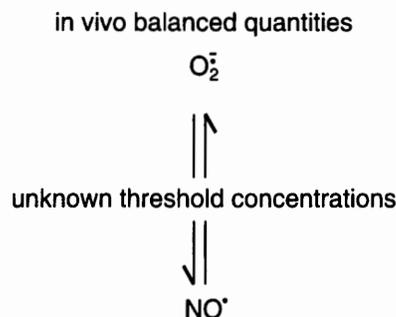


Fig. 1. Schematic of a study of free-radical species.

brane and enter cells; therefore, low-molecular-weight mimics of SOD enzymes that can cross cell membranes are important. This is because free radicals produced during oxidative bursts on exposure to ionizing radiation cannot be prevented by the concentrations of SOD enzymes normally available inside the cells.

The manganese-desferral complexes are SOD mimics, which have been reported to have protective effects against oxidative stress in cells. However, our EPR results (fig. 2; Gray and Carmichael 1992) showed that solutions of these complexes are 3-4 and 2-3 orders of magnitude less active than CuZnSOD and MnSOD or FeSOD, respectively (table 1). The results also suggest that the reactivity towards O_2^- in solutions of these complexes originates from the free Mn^{+2} present and not from the desferral complexes.

Because protection against oxidative stress is important to ameliorate radiation injury, the toxicity of aminothiol radioprotectors was initiated prior to their use. Pathological evaluation of WR-151327 in male mice was found to be protective to the intestine but extremely toxic to the testes (Steel-Goodwin et al., 1992). Initial studies on WR-1065, WR-2721, and WR-3689 showed similar effects as reported by Steel-Goodwin et al. in May 1992 at the Association of Clinical Scientists Meeting in Syracuse, N.Y. Furthermore, EPR/spin trapping studies in the intestine show that WR-1065 induces the production of the free radical NO^* as reported by Steel-Goodwin et al. in October 1991 at the 2nd International Meeting on the Biology of Nitric Oxide in London, United Kingdom.

Results from experiments in the intestine also implicate NO^* in normal gut peristalsis as reported by Steel-Goodwin et al. in June 1992 at the Nitric Oxide Implications for Drug Research Meeting in

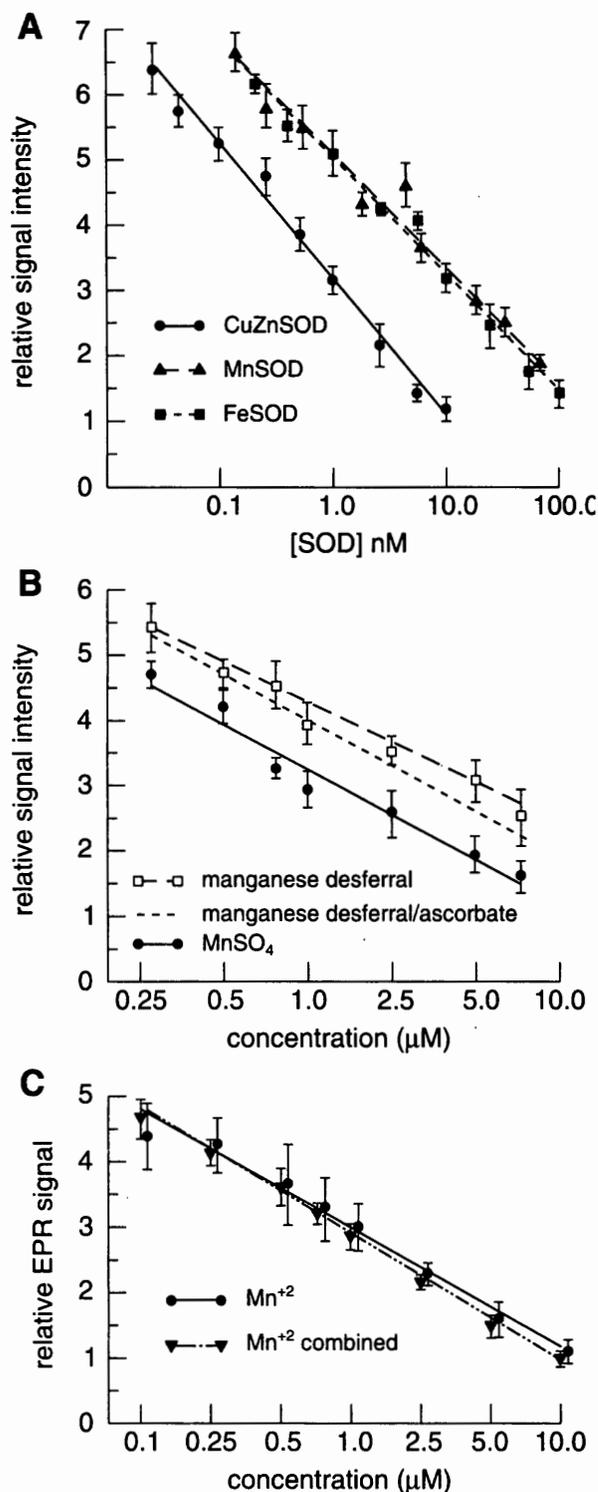


Fig. 2. Kinetic profiles of (A) superoxide dismutases; (B) equal concentrations of manganese desferral, manganese desferral/ascorbate, and MnSO₄ complexes competing with the spin trap DMPO for superoxide; and (C) combined data for manganese desferral and manganese desferral/ascorbate solutions adjusted to have equal concentrations of Mn²⁺ also equal to the concentration of Mn²⁺ from an MnSO₄ solution.

Table 1. Rate constants for the reaction of superoxide with superoxide dismutases, and superoxide with Mn²⁺ from manganese desferral, and manganese desferral/ascorbate complexes and with Mn²⁺ from MnSO₄.

Compound	Rate constant (M s ⁻¹)
CuZnSOD	6.4[4.90, 7.9]x10 ⁹
FeSOD	6.6[5.50, 7.7]x10 ⁸
MnSOD	6.8[5.40, 8.2]x10 ⁸
Mn ²⁺ standard	2.3[0.46, 4.1]x10 ⁶
Mn ²⁺ complexes	2.9[2.20, 3.6]x10 ⁶

Philadelphia, Pa. The intestine contains smooth muscle, an extensive nerve supply, and a large vascular bed. NO[•] plays a central role in the biochemistry of all these systems. Therefore, in the vascular system, EPR/spin trapping was used to study the existence and biological role of L-arginine/NO[•] pathway in human platelets (Pronai et al., 1991).

EPR and spin trapping techniques were also applied to study reactions between active oxygen and nitrogen species of possible importance in biology. Peroxynitrite (OONO[•]) is generated by the reaction of O₂⁻ with nitric oxide and by reaction of their respective biological degradation products, H₂O₂ and nitrite. Indirect evidence has suggested that this intermediate decomposes, forming hydroxyl radicals and nitrogen dioxide. Our initial spin trapping results have detected the formation of hydroxyl radicals in the decomposition of peroxynitrous acid (HOONO) as reported by Carmichael et al. in November 1991 at the 3rd International Congress on Spin Trapping and Aminoxyl Radical Chemistry in Kyoto, Japan.

In conclusion, this project addresses, using EPR techniques, the fundamental mechanisms of radiation damage (free radicals) in biological systems. Effective means of protection against the effects of ionizing radiation are also studied by EPR techniques.

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Size of hemopoietic stem cell compartment in various species

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Project 04620

In order to extrapolate radiation effects on hemopoietic stem cells (HSC) from small experimental animals to humans, we must know the total number of HSC in both species. Previously, it was thought the marrow concentration or frequency of HSC in both species was the same. If so, the average 70-kg human, being approximately 2,800 times as large as the 25-g mouse, would be expected to have a total HSC population 2,800 times that of the mouse. Yet, new methodologies for measuring and marking HSC have generated enough data to indicate that a few or perhaps one HSC can totally repopulate the marrow of lethally irradiated mice and humans. Therefore, the concept of invariant marrow HSC frequencies among species is being questioned for there is no compelling reason to believe that a human would require or benefit from maintaining a relatively large, dormant

HSC population throughout a long life span. It is our purpose to determine whether the marrow HSC frequency of the human and the mouse is proportional or inversely proportional to body weight. Such information is vital to the military for predicting survival of irradiated humans in various combat scenarios.

We are establishing a method whereby, in both small and large animals, the number of HSC surviving both uniform and nonuniform irradiation can be accurately measured by an assay based on the theory of X-chromosome inactivation (Lyon, 1974; Micklem et al., 1987). Briefly, random inactivation of one of the two X-chromosomes in the cells of placental mammalian females during early development ensures that females have the same dose of X-chromosomes as males. If a female is heterozygous for the X-chromosomes and one X-chromosome is inactivated, then most cell compartments, including the HSC compartment and its descendants are, in fact, mosaics of cells that can be identified as either "A" or "B" cells, depending upon the clone from which they were derived. In the case of HSC, the total number of embryonic clones from which adult HSC are derived is given by the binomial formula $n=p(1-p)/s^2$, where p is the probability of an HSC being "A", $1-p$ is the probability of a "B" HSC, and s^2 is the variance of p . Solving the equation gives n , or the total number of embryonic cells from which HSC are derived. Because p can be determined experimentally, the number of HSC surviving uniform and nonuniform neutron or gamma radiation can also be calculated as a function of dose for most mammals including humans.

During fiscal year 1992, female mice heterozygous for the X-chromosome-linked PGK alloenzyme were specially bred for us at Charles River Laboratories, Willington, Mass. We have determined the individual p values for more than 180 of these female PGK-1^{a/b} mice. Average p for the PGK-1^a form was determined to be 0.74 ± 0.12 (mean \pm SD) and the variance was 0.0146, giving an n of 13.03. Thus, at that point in ontogeny dur-

If a female is heterozygous for the X-chromosomes and one X-chromosome is inactivated, then most cell compartments, including the HSC compartment and its descendants are, in fact, mosaics of cells that can be identified as either "A" or "B" cells, depending upon the clone from which they were derived.

ing which all embryonic cells underwent X-chromosome inactivation, 13 clones were committed to hemopoiesis. The cumulative fit as a function of percent of PGK-1^a is shown in figure 1. For comparison, a binomial distribution with the parameters $n=13$, $p=0.74$ is also shown.

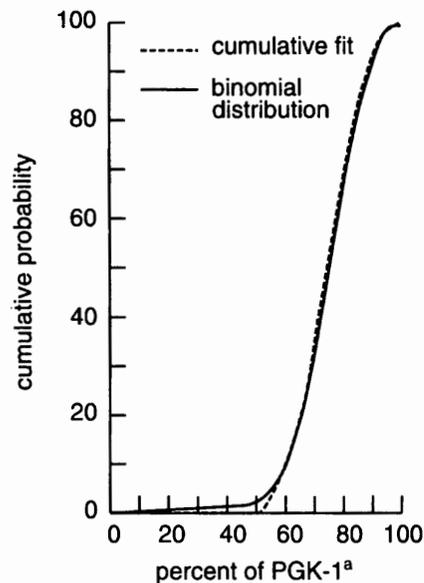


Fig. 1. Cumulative distribution of mean percent PGK-1^a observed in 184 female C3H/HeHa mice as compared with corresponding cumulative distribution predicted by a binomial model with $p=0.74$ and $n=13$.

Because preirradiation values for p from all the irradiated mice have been determined, it is possible to predict the probability of observing only the PGK-1^a or -1^b forms postirradiation. According to binomial theorem, it would be p^n for the PGK-1^a form and $(1-p)^n$ for the PGK-1^b form, where n is the number of surviving HSC. Groups of mice were irradiated to a 6-, 7-, and 8-Gy total-body dose from the AFRRI ⁶⁰Co source. Percent PGK-1^a or -1^b will be determined and the number of surviving stem cells calculated as stated above.

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- McCarthy KF, Hale ML, Craw PD (1992) Size of the rat hematopoietic long-term repopulating cell population. *Experimental Hematology*

The protocol includes two complementary efforts. The first is the clinical follow-up of Desert Storm patients known or suspected to have imbedded DU fragments, DU-contaminated wounds, or significant amounts of inhaled depleted uranium. The second is the conduct of research into the toxicological and radiological effects of this unique exposure modality.

Specifically, the protocol provides for the following actions:

- Early detection of DU-related abnormalities, followed promptly by efficacious treatment. This action will provide the scientific data required to fairly settle compensation claims.
- Treatment recommendations that will provide a firm clinical basis for decisions regarding fragment removal and efforts to reduce the uranium in the body.
- Quantification and documentation of the toxicological (heavy metal toxicity) and radiological (cancer and tissue necrosis) risks associated with imbedded DU fragments. This action will involve the use of in vivo and in vitro techniques to measure and document uranium levels in each soldier. It will determine the parameters and models needed to translate uranium levels in the body into estimates of increased cancer risk from DU exposure. It will compare the body's response to DU fragments with that to non-DU fragments to determine whether clinically significant differences exist due to either the chemical or radiological properties of depleted uranium. It will determine the risk of chronic kidney toxicity due to the long-term, chronic exposure to elevated levels of uranium.

We do not know conclusively how many soldiers were wounded by DU fragments in the incidents cited. However, an initial check of U.S. Army records revealed 22 soldiers who have imbedded fragments that might be DU and 13 who were wounded and hospitalized but were not identified as having shrapnel. We evaluated 2 of the 22 with imbedded fragments and found that both have elevated levels of uranium in their urine.

The remaining soldiers either were not wounded during the incident or had minor wounds that were treated in the field. The latter two sets of soldiers might have DU contamination from inhaled uranium, from wounds that were treated in the field, or from minor fragmentation wounds that either were not noticed or did not require extensive treatment.

Animal research will seek information not available through patient monitoring. The research will answer, for instance, the following questions: What is the threshold of uranium for long-term kidney toxicity? What changes in kidney function and pathology result from chronic exposure to elevated levels of uranium in the body? How does radiation affect the encapsulation process as a function of time in the body? What are the effects on neural tissues of the long-term exposure to low-dose-rate alpha and beta radiations?

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Outreach Program

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Program goals

- Formulate and recommend to the AFRRRI director short- and long-term plans, policies, and programs, based on the individual military qualitative research requirements and joint service operational requirements concerning radiobiology and related areas.
- Maintain lines of communication and cognizance, regarding technology transfer activity, with the Radiation Policy Division and other elements of the Defense Nuclear Agency (DNA).
- Provide oversight and coordination for liaison functions and communications regarding radiobiology and the application of medical techniques to mitigate undesirable effects of radiation exposure.
- Coordinate and integrate radiobiological data produced by AFRRRI's scientific research programs to ensure effective transfer of information to the military.

- Plan, establish, and manage comprehensive training programs to educate military and other government personnel about the operational and medical effects of nuclear weapons and ionizing radiation.
- Establish and manage an emergency response team to provide advice and consultation on state-of-the-art protocols for the treatment of radiation injuries.
- Improve the productivity and effectiveness of selected demonstration laboratories.

Requirement

Our radiobiology research must be of the highest quality, as judged by nonmilitary scientific peers, and must be usable in the military operational arena. Such targeted research requires a dynamic process that identifies the needs of the target community and provides the mechanisms for dissemination of research progress and results. At the same time, we must endeavor to improve productivity and effectiveness of laboratory operations.

To those ends, AFRRRI's liaison functions and communications must encompass DNA Headquarters, Department of Defense (DoD) agencies and activities, other government and nongovernment agencies, and the scientific community.

Strategy

DNA, AFRRRI, and the military services collaborate to determine whether new requirements of military medical and operational programs can be accomplished within available assets and the AFRRRI mission. The requirements are addressed through integrated, interdepartmental research pro-

jects. Those findings are disseminated to health care providers, disaster preparedness personnel, and operational planners through the Medical Effects of Nuclear Weapons Course and other seminars, conferences, and workshops.

Advice and consultation on emergency response to radiation accidents are provided by the Medical Radiobiology Advisory Team. AFRRRI's Military Requirements and Applications Department provides health physics, medical, and site

MENW Course

During fiscal year 1992, AFRRRI staff members presented lectures for the Medical Effects of Nuclear Weapons (MENW) Course to 226 attendees (131 in February and 95 in August) at the Ramada Renaissance Hotel, Herndon, Va.

Modified presentations were given to residents at the National Naval Medical Center, Bethesda, Md., in October 1991 and March 1992. Other individualized courses were presented as follows.

<u>Location</u>	<u>Number of students</u>	<u>Location</u>	<u>Number of students</u>
Dhahran Air Base Dhahran, Saudi Arabia	52	Royal Air Force Lakenheath Lakenheath, United Kingdom	46
Naval Aerospace Medical Institute Pensacola, Fla.	103	10th MEDLAB Sembach Air Base, Germany	65
Charleston Naval Base Charleston, S.C.	64	Oakland Naval Hospital Oakland, Calif.	55
Naval Undersea Medical Institute Groton, Conn.	36	Fort Lewis Tacoma, Wash.	65
U.S. Air Force Academy Colorado Springs, Colo.	72	Fort Lewis Reserve Component Tacoma, Wash.	66
Camp Casey Tonduchon, South Korea	38	Army Nurse Corps Fort Knox, Ky.	69
121st Evacuation Hospital Seoul, South Korea	49	USAF School of Aerospace Medicine Brooks AFB, San Antonio, Texas	32
Hickam Air Force Base Honolulu, Hawaii	40	Fleet Marine Medical Officers Naval Air Station, Pensacola, Fla.	25
Fort Bragg Fayetteville, N.C.	77	School of Military Engineering Holsworthy, New South Wales, Australia	<u>29</u>
7th MEDCOM Vilseck, Germany	42		
		Total	1,025

restoration support to the DNA Advisory Team as well as through NATO Standardized Agreements, handbooks, and peer-reviewed articles in scientific journals.

AFRRI continues to participate in the Laboratory Demonstration Program (LDP) established in November 1989 by the Office of the Deputy Secretary of Defense. The Institute, approved in September 1990 as one of a select group of DoD demonstration laboratories, maintains strong representation in the program to evaluate recommended changes in administration- and management-targeted activities, including regulatory and legislative changes.

The primary areas under continuing study are personnel management, facility modernization, and contracting. Personnel management goals include increased authority for the laboratory director to manage workforce-to-budget workload (exempt from freezes, ceilings, etc.), to use direct hire au-

thority for all civilian grades/career fields, to classify and appoint senior technical specialists, and to institute pay banding to link pay to performance. Facility modernization goals involve a long-range renewal plan and an increase in military construction from \$300 thousand to \$1 million. Contracting goals are to streamline the contract format and increase the small purchase threshold from \$25 thousand to \$100 thousand.

AFRRI Director Robert L. Bumgarner, CAPT, MC, USN, serves as a voting member of the LDP Executive Panel, which reports directly to the Office of the Director of Defense Research and Engineering. Dr. Mildred A. Donlon serves as an alternate voting member and is chairman of the Implementation Subpanel, which is responsible for the preparation of the LDP annual report for the Office of the Deputy Secretary of Defense as well as for the LDP implementation plans for all program laboratories.

Project members' publications/presentations, fiscal years 1991-1992

Journal articles

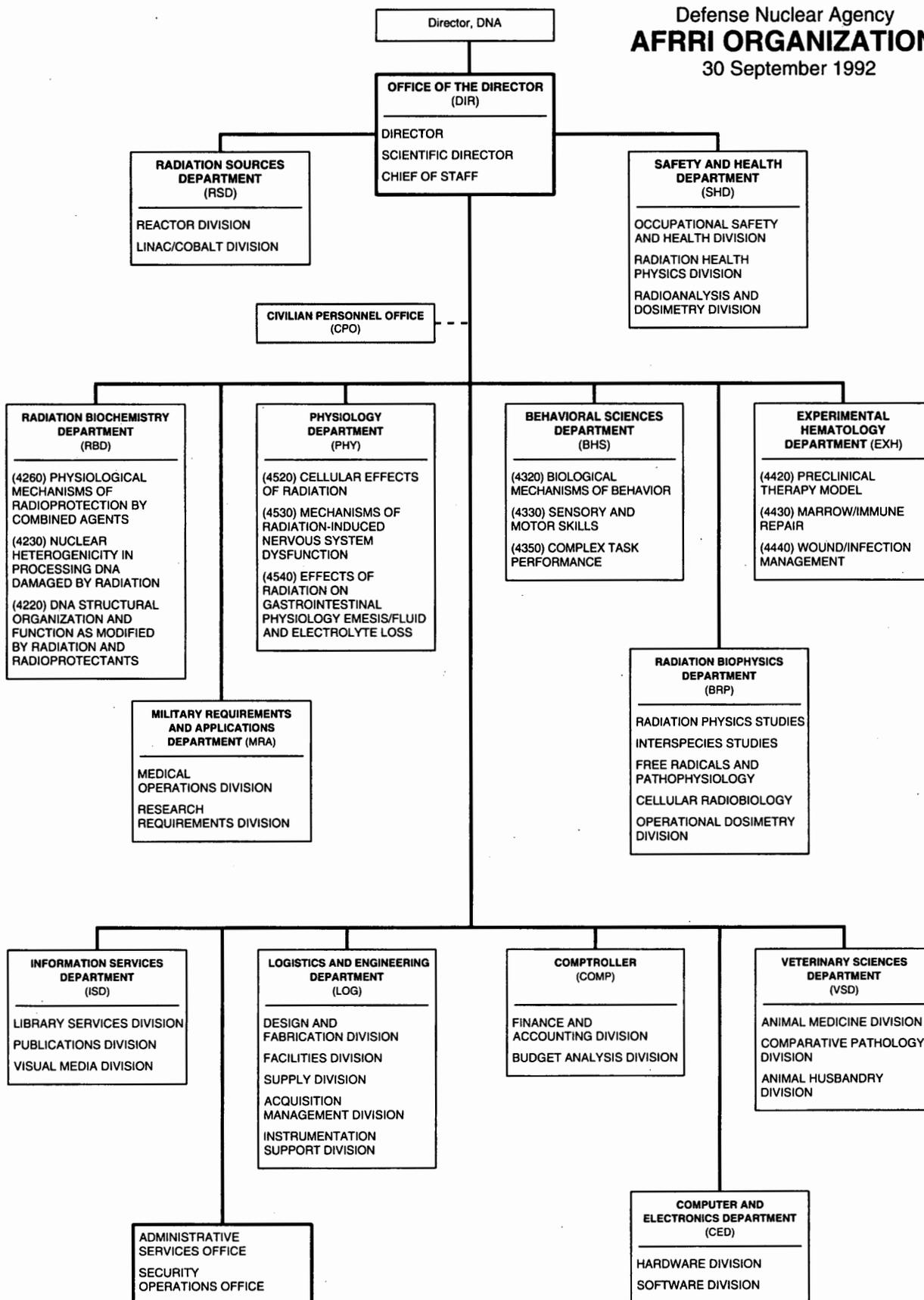
Browne D, Weiss JF, MacVittie TJ, Pillai MV
(1992) Protocol for the treatment of radiation

injuries. *Advances in Space Research*
12(2):165-168



The Armed Forces Radiobiology Research Institute complex.

Defense Nuclear Agency
AFRRI ORGANIZATION
 30 September 1992



Research Support Activities

The more than 100 scientists and technicians at AFRRRI are supported by almost 200 military and civilian personnel. The support staff's contributions include the operation of radiation sources, the maintenance of an accredited laboratory animal facility, the development of computerized experimental designs and information management programs, and the acquisition and dissemination of research data.

Others provide support in the areas of health and safety monitoring; facilities, equipment, and supply services; financial management; personnel and security oper-

ations; and publications and graphics services.

Year round at AFRRRI, safety and health specialists oversee the safety of all AFRRRI operations. They issue personal dosimetry for employees and visitors; oversee the handling, use, and disposition of radioactive and chemical substances; maintain environmental monitoring programs; and develop safety manuals and training programs. For example, during the past year, training covered blood-borne pathogens, chemical hygiene, and emergency response procedures. AFRRRI also serves as the Defense Nuclear Agency



Support Services Specialist Michelle Glasser and Administrative Support Assistant Cynthia Burrows confer on a request for logistical support.

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Administrative Officer

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Cynthia L. Phillips

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Marion Golightly

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Rennett Goodman

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Robinson Colon

**Radioanalysis and Dosimetry
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**Radiation Health Physics Division
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Accounting Technicians

Gwendolyn S. Moore

Mary E. Williams

Voucher Examiners

Joyce A. Wilson

Meredith Acker-Ford

(DNA) resource for occupational safety and health guidance and support. The first DNA Occupational Safety and Health Meeting was conducted Jan. 16-17, 1992.

During fiscal year 1992, logistics experts acquired, within 24 hours, mandatory desert sand uniforms and equipment for the AFRRRI team who presented a course in Saudi Arabia on the medical effects of nuclear weapons. Facilities experts coordinated the completion of a 3,000-square-

foot building addition and the wiring for the computer system's local area network.

Facilities specialists routinely provide basic services; and logistics specialists buy, store, and inventory goods ranging from reactor consoles to office supplies. Instrumentation specialists not only maintain and calibrate an array of laboratory recording devices but also design and build customized equipment for experiments.



Physical Science Technician Joan A. Smiley prepares to analyze environmental samples for radioactivity while HM2 John P. Hokenson, USN, and Emma L. Kephart, a health physicist, discuss radioanalysis results.



Engineering Technician Franklin M. Sharpnack uses a precision vernier caliper to meet measurement specifications for a prototype research device.

Fiscal management specialists, in addition to coordinating the internal management control program, carry out detailed planning, programming, budgeting, and accounting.

Personnel and administrative specialists oversee staffing, training, correspondence, and record keeping while security experts monitor classified operations.

Photographers and illustrators prepare visual materials and art for printed media, scientific presentations, and displays. The editorial staff produces reports and manuals, coordinates the publication of meeting proceedings, and reviews manuscripts for publication in scientific journals.



Engineering Technician Jimmie L. Powell operates a lathe in the production of a prototype research device.

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LTC, MS, USA

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***Design and Fabrication Division
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Franklin M. Sharpnack

Donald N. Stevens

Jimmie L. Powell

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Electrician

W. Michael Rentzell

Maintenance Mechanics

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P. Ray Rowland

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Scientific-Technical Photographer

David H. Morse

Audiovisual Production Specialist

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Photographer

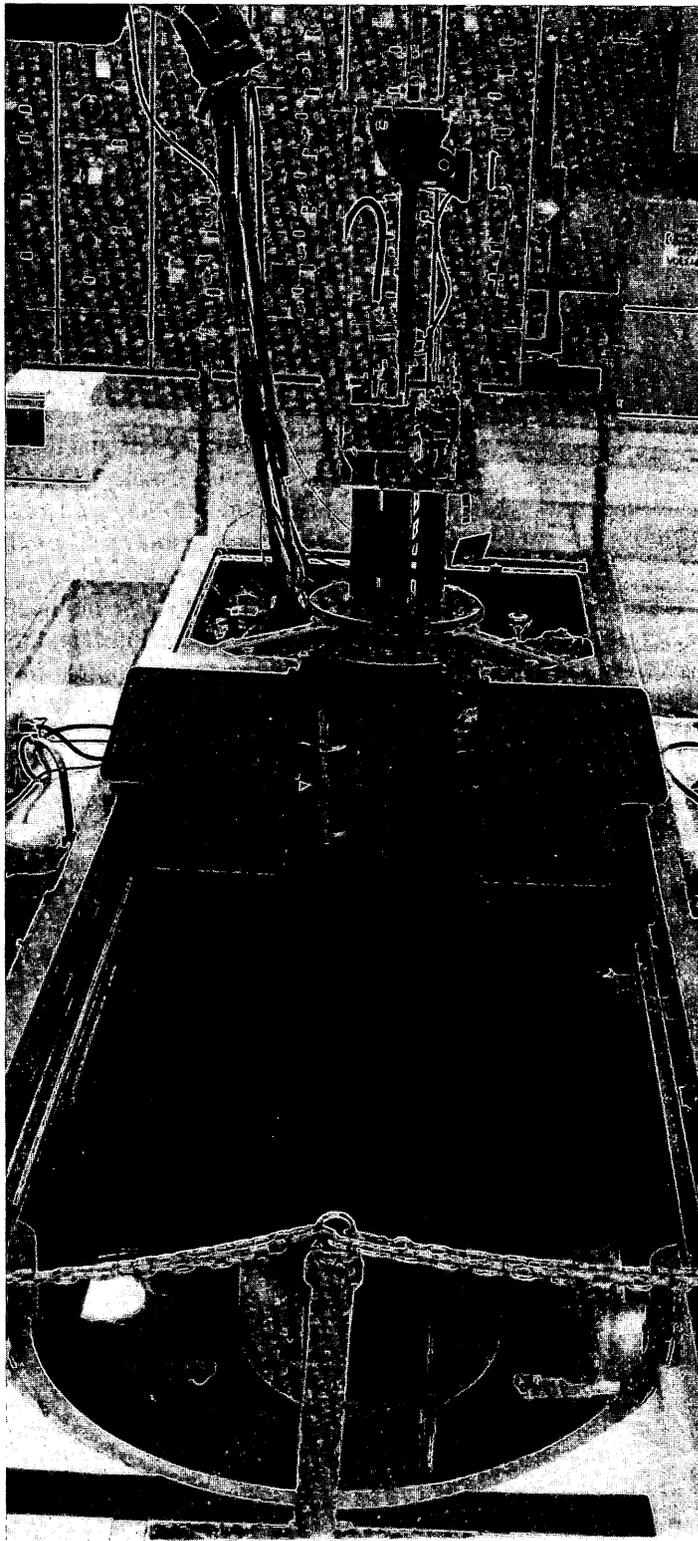
David Bartky

Illustrators

Mark A. Behme

Guy R. Bateman, B.S.

Darrell Z. Grant, B.A., A.A.



The support structure of the Mark-F TRIGA, a medium-sized research reactor, is designed for a moveable core.

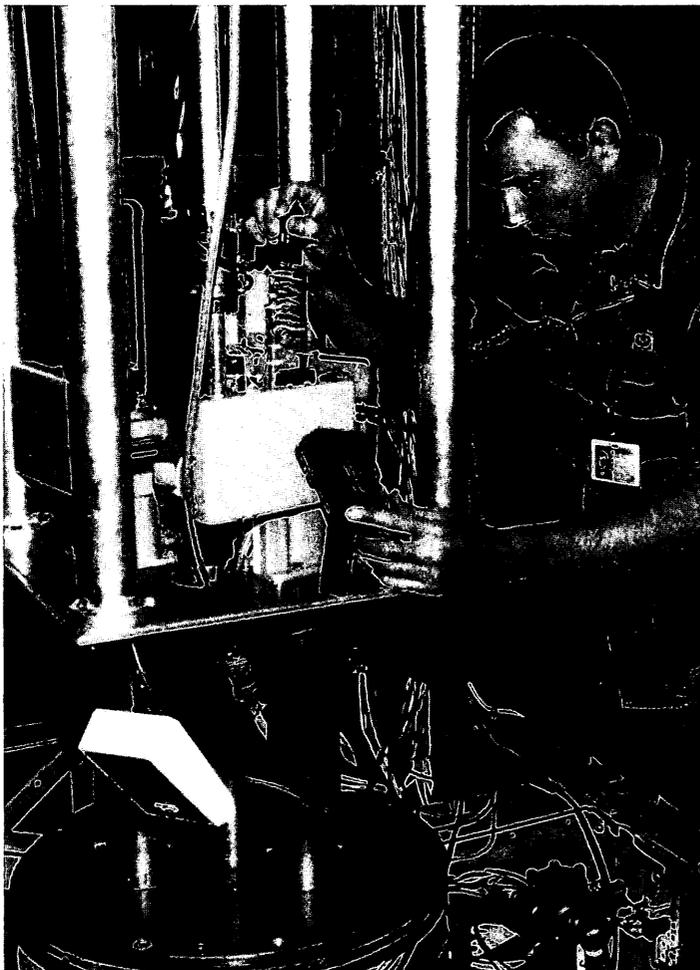
Research reactor's new exposure cave using bismuth allows modified spectrum

A number of unique radiation sources provide AFRRRI's scientists with a variety of radiations, energies, and dose rates. A medium-sized research reactor (Mark-F TRIGA), a 400-thousand-curie gamma irradiation facility (cobalt-60), and a 4,200-curie therapeutic irradiator (Theratron-80) are licensed by the Nuclear Regulatory Commission. In

addition, we have a traveling microwave high-energy linear accelerator (LINAC) and a 320-kVp industrial x-ray machine (Phillips).

Research reactor

A new exposure cave using bismuth was placed in operation in



SFC Michael Laughery, USA, measures the resistance of a reactor control rod magnet.

Radiation Sources Department Head

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CAPT, MSC, USN

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Carol J. King

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Robert A. George, B.S.

Harry Spence, B.S.
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Michael E. Laughery, A.A.
SFC, USA

Reactor Operator Trainee

Stephen I. Miller., B.S.

LINAC/Cobalt Division Chief

Arthur B. Webb, Ph.D.
LTC, MS, USA

LINAC Facility Director

Mark Gee, B.S.

Cobalt Facility Director

Ernest Golightly

Cobalt Operator

William C. Wilson, B.S.
HMC, USN

fiscal year 1992. This allows the reactor group to modify the spectrum by removing neutrons without the resulting production of capture gammas.

In addition to supporting AFRRRI researchers during fiscal year 1992, the reactor produced krypton-85 gas for use in a study of tissue inert gas exchange kinetics conducted by the Naval Medical Research Institute, and it supported a University of Maryland study of the inverse dose rate effects of neutrons.

The Mark-F TRIGA has a pool, a movable core, and dry exposure facilities. It can run at a steady state of 1 megawatt or pulse at up to 2,500 megawatts in about 0.1 second. The acronym "TRIGA" denotes the manufacturer, Training, Research, Isotope, General Atomics.

The reactor's gamma/neutron ratio can be varied from 1:20 to 20:1 by means of shields and absorbers placed in the exposure rooms. Operators can alter the energy of the neutron beam by moving the core, within its pool, in relation to the exposure room. Exposure rates can be varied from about 0.1 rad/minute to 100 thousand rads/minute/pulse.

The two exposure rooms provide ample space for dry experimental setups with fast or thermal neutron-weighted spectra. The reactor has been fitted with a small-animal extractor to provide a 20-second turnaround time versus the 45 minutes needed to cycle the large concrete plug-doors.

This system uses a motor-driven pulley to handle experiments of approximately 8 inches. The core experiment tube is used



MSG Harry Spence, USA, prepares to deliver a radiation dose to a biological experiment.

to attain high exposures for small experiments.

The reactor's portable beam tube system consists of aluminum tubes suspended in the reactor containment pool. The system produces a controlled beam of radiation, which allows the irradiation of selected anatomical areas. Operators use filters and lenses to vary the degree, character, and precision of the radiation.

Linear accelerator

The LINAC, designed and assembled by Varian Associates be-

tween 1965 and 1968, provides a powerful, flexible source of high-energy electrons, high-energy bremsstrahlung (x rays), and neutrons. Applications include radiobiology and radiochemistry studies as well as those concerning electromagnetic pulse and radiation. The accelerator is also used to sterilize tissues and equipment and to assess radiation damage to electronic semiconductor circuits and devices.

Since the LINAC has six accelerating sections that can be powered by up to four klystron microwave amplifiers, a variety of machine configurations can be

used to provide electron energies continuously variable from 10 to 54 MeV.

If a target requires a greater penetration depth than that of high-energy electrons, the beam can be converted to bremsstrahlung (x ray) and used in those cases where the mean free path or range of electrons is too low. The average energy for bremsstrahlung is 3-MeV photons when an 18-MeV electron beam is incident on the converter. With the proper converter in place, the LINAC can produce 8×10^{15} neutrons per second.

Cobalt-60 facility

The cobalt-60 facility, which opened in 1969, can provide large, uniform gamma-ray fields at variable dose rates with flexible configurations in both unilateral and

bilateral irradiation modes. The facility, which is below ground in the AFRRRI complex, has a 35-square-foot, 25-foot-8-inch-high exposure room. The combination of massive reinforced concrete and earthfill outside the cobalt exposure room provides shielding for up to 500 thousand curies.

A variety of source combinations can be loaded on the facility's two elevators while they are submerged. The exposures deliverable when the elevators are raised from the water can range from rads/hour to 20,000 rads/minute. The radiation fields are uniform and vary inversely with the dose rate delivered.

Theratron-80

AFRRRI has an AECL Theratron-80 therapeutic irradiator that

provides a backup for the cobalt-60 facility as well as exposure capability for cellular research. This unit can provide from 1 to several hundred rads/hour over limited field sizes. The Theratron-80 also provides a uniform field traceable by dosimetry to the National Institute of Standards and Technology.

Industrial x-ray machine

A Phillips industrial x-ray machine, a water-cooled device that can be run indefinitely, provides x rays of from 40 to 320 kVp. Depending on field size, the output can be varied from a few to 7,000 rads/minute. Like the Theratron-80, it is used mainly for cellular work and as a backup for the cobalt-60 facility.

Department's publications/presentations, fiscal years 1991-1992

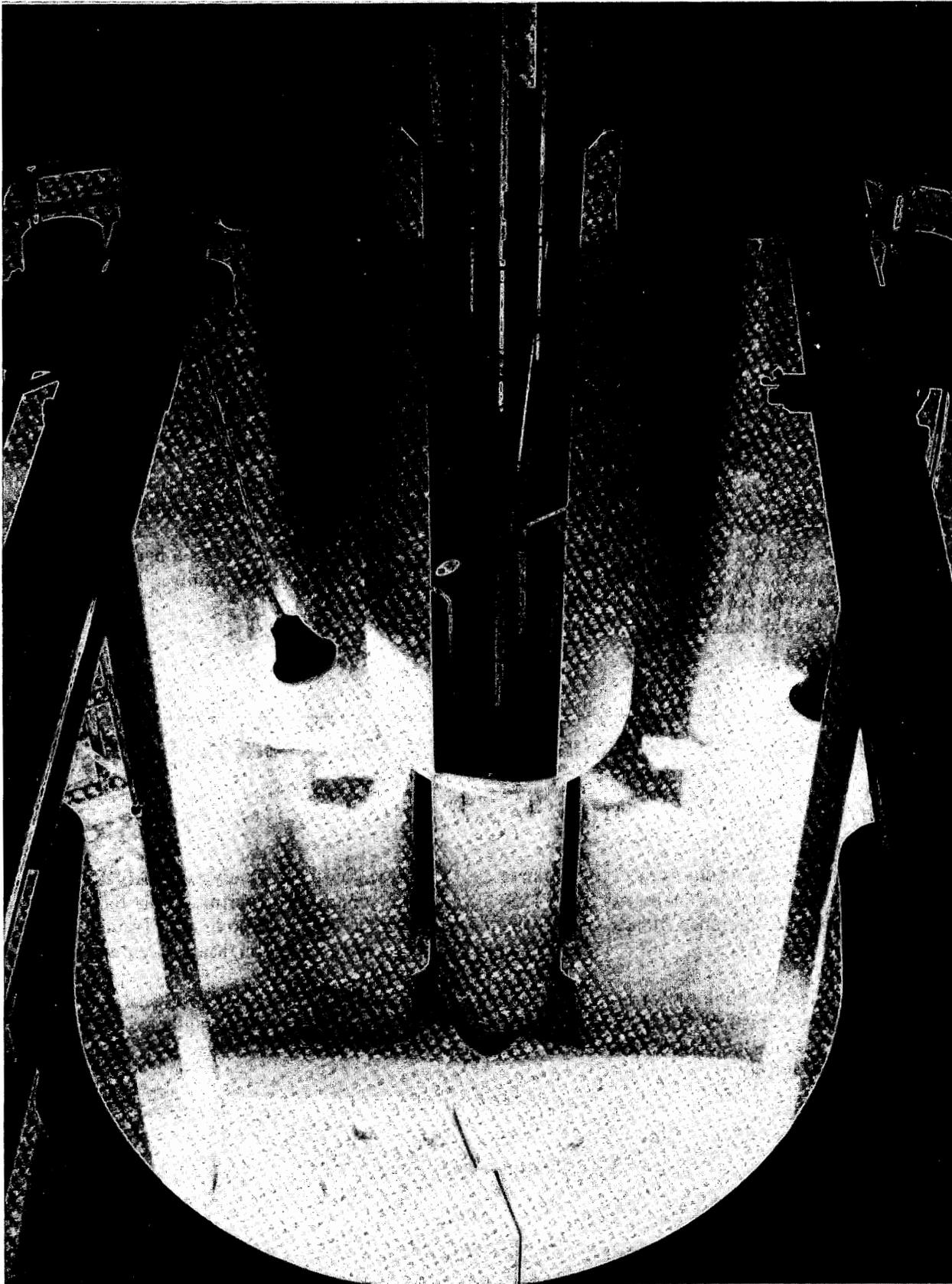
Abstracts

George R. An ongoing reactor project to eliminate algae and bacteria from the reactor pool. U.S. TRIGA Users Conference, Ithaca, N.Y., May 1992

Moore M. Nuclear technology for nondestructive investigation of historic coffins at St. Mary's City, Md. U.S. TRIGA Users Conference, Ithaca, N.Y., May 1992

Moore M, Owens C. Overview of AFRRRI and reactor supported experiments in biological research. Test, Research, and Training Reactor Conference, Boston, Mass., October 1991

Owens C. Procedures and results of AFRRRI fuel-follower control rod installation. U.S. TRIGA Users Conference, Ithaca, N.Y., May 1992



The core and pool of the Mark-F TRIGA research reactor viewed from atop the support structure.

Operational dosimetry

The Operational Dosimetry Division of the Radiation Biophysics Department provides high-quality, state-of-the-art dosimetry to support AFRRRI's radiobiology experimentation and modelling efforts. Our approach includes quality assurance, radiation field characterization, and dosimetry research.

Quality assurance

AFRRRI's dosimetry quality assurance program is a combination of rigorous internal audits and participation in national and international dosimetry intercomparisons. The intercomparisons are conducted with the National Institute for Standards and Technology (NIST), Gaithersburg, Md., and the University of Texas M.D. Anderson Cancer Center (MDACC),

Houston, Texas. These intercomparisons are performed on our electron linear accelerator (LINAC), Theratron-80 therapeutic irradiator, and cobalt-60 facility. Intercomparisons with NIST and NATO involve mixed field neutron/gamma radiations from AFRRRI's TRIGA reactor and californium sources.

The results of our participation in fiscal year 1992 intercomparison experiments are shown in figure 1.

Radiation field characterization

We use a wide range of dosimetry systems to quantify the radiation exposure for the experiments conducted at AFRRRI. Our systems provide for passive and real-time

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Leon Goodman

Betty Ann Torres

James Myska

Radiological Physicist

Henry Gerstenberg, M.S.

Physical Science Technician

Gregory Johnson

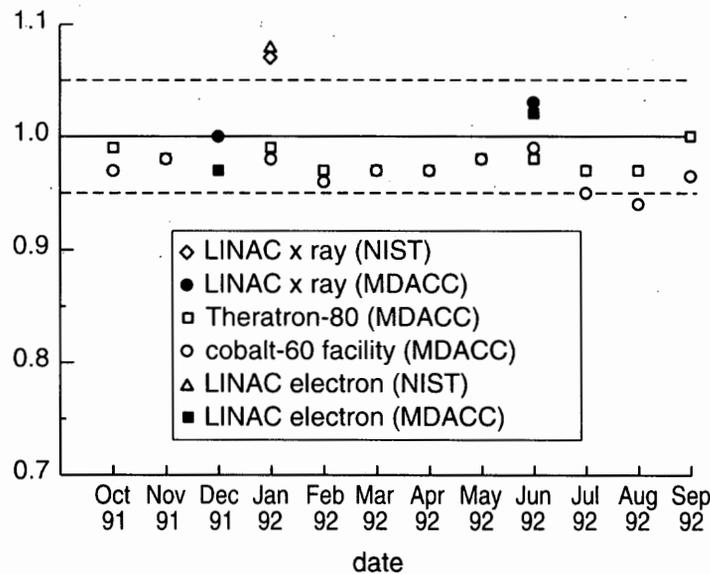


Fig. 1. Results of MDACC and NIST intercomparisons. Each ordinate is the ratio of the AFRRRI-reported result to the standard value. The dotted lines represent the range of values considered acceptable under the protocol for these intercomparisons (5%). Each MDACC intercomparison used the LiF thermoluminescent dosimeter (TLD) 100. Each NIST intercomparison used the Fricke chemical dosimeter.

measurement of the dose, dose rate, and radiation field quality in each of our sources, using ionization chambers, tissue equivalent proportional counters (TEPCs), and a variety of TLDs.

Our ionization chambers and TLDs are the primary measurement devices for our LINAC and cobalt-60 gamma ray sources. Our ionization chamber measurements for these sources are conducted in accordance with the American Association of Physicists in Medicine protocol TG21. The wide variety of ionization chambers on hand enable us to measure the dose from photon sources whose energy varies from several keV to the high-energy bremsstrahlung photons that are generated in our LINAC. We can provide depth dose information for both photon

and electron fields, using several available phantoms.

Our neutron and mixed neutron and gamma dosimetry uses the standard paired chamber technique to obtain estimates of the total dose and estimates of the fraction of the total dose attributable to each of the components of the exposure field. Both the photon and neutron spectra have been well characterized. These spectra are readily available and well documented. We monitor changes in these values through the use of activation foils and NIST-designed fission chambers. We have a wide range of tissue equivalent phantoms for depth dose measurements in these fields.

Our microdosimetric capability allows us to monitor the quality

of the radiation fields for all our sources by using a biologically significant parameter (lineal energy). This system has been successfully used in several of our neutron experimental setups.

Research

Our research efforts focus on the development of new dosimetry systems and exposure configurations to meet the needs of the experiments at AFRRRI. In collaboration with NIST, we are investigating the use of radiochromic dye film and alanine pellets in mixed neutron and gamma radiation fields. Several experiments have been conducted, and the results are being analyzed.

Division's publications/presentations, fiscal years 1991-1992

Gaiter SL. Criticality accident dosimetry — use of the Navy's nuclear criticality dosimeters. 33rd Navy Occupational Health and Preventive Medicine Workshop, Virginia Beach, Va., March 1992

Gerstenberg H. Depth dose measurements of 200 MeV protons using alanine and other dosimeters. 10th International Conference on Solid State Dosimetry, Washington, D.C., July 1992

Gerstenberg H. Some neutron-source characteristics used to model biological response to neutron radiation. 11th International Symposium on Microdosimetry, Gatlinburg, Tenn., September 1992

Musk JH. Thermoluminescence characteristics of aluminum oxide. 10th International Conference on Solid State Dosimetry, Washington, D.C., July 1992

Accredited program provides purpose-bred research animals

AFRRI's animal care program has continued since 1984 to maintain accreditation with the American Association for Accreditation of Laboratory Animal Care (AAALAC), a nonprofit corporation that encourages the highest standards for the care and use of laboratory animals.

During fiscal year 1992, our program consisted of off-site breeding colonies and a 32,000-square-foot in-house facility. The program provided nine species of disease-free, purpose-bred research animals. Of the 60,969 animals used, 98% were rodents (fig. 1).

Our veterinarians oversee administrative operations, animal husbandry, comparative pathology, and animal medicine. They consult with our scientists on ani-

mal issues, veterinary medical and surgical services, necropsy, and pathological tissue evaluations. In addition, they work with the organization's Institutional Animal Care and Use Committee during the research protocol review process.

Our animal care specialists and other support personnel are critical to the operation and maintenance of our program. They order and monitor the animal inventory by species, screen and quarantine incoming animals to maintain health records, and provide daily care and feeding. Some also provide clinical, microbiological, and histological services; others maintain a clean and healthful animal facility so as to minimize disease and provide a safe work environment.

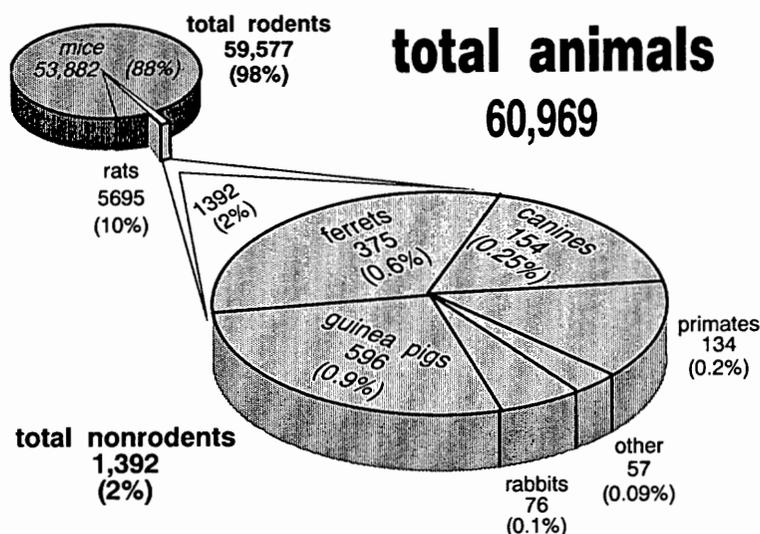


Fig. 1. Purpose-bred research animals used during fiscal year 1992. The "other" category included, for example, lobsters and frogs.

Veterinary Sciences Department Head

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Comparative Pathology Division Chief

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Mary J. Morgan, LAT

Cesar Reyes

Ronald Caho, ALAT

Luis Perez

Antonio Lopez

David Best

Cage Wash Laborers

Leroy Taylor

John Ebaugh

Douglas Anderson

Gregory Jefferson

The American Association for Laboratory Animal Science certification program includes Laboratory Animal Technologist (LATG), Assistant Laboratory Animal Technician (ALAT), and Laboratory Animal Technician (LAT).



Dr. Roy Vigneulle and Lisa Grab exteriorize rat intestines in preparation for irradiation.

Chapman Library collections include those of the Atomic Bomb Casualty Commission

The William H. Chapman Library is a biomedical research library that supports the information requirements of AFRRRI's research mission.

The library's special collections include extensive holdings from the Atomic Bomb Casualty Commission, currently called the Radiation Effects Research Foundation, and the U.S. Naval Radiation Defense Laboratory, now decommissioned. The library's principal subject area is the biomedical effects of radiation on

human and animal cells; however, it acquires materials on diverse subjects, such as behavioral science, cancer, hematology, immunology, pathology, physiology, psychology, veterinary medicine, and management.

Scientific and technical information is available to military and civilian researchers through 10,000 books, 20,000 bound periodicals, 50,000 technical reports and articles in print or on microfiche, and 200 audiovisual presentations (audio cassettes, slide sets,

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Cataloging Librarian
Martha R. Harris, B.A., M.A.
M.L.S.

Circulation Technician
Myron K. Allman



Dr. Elsa Schmauder-Chock selects a book from the stack while, at the computer, Dr. Ruth Neta, Dr. K. Sree Kumar, and Rita Harding use the MEDLINE data base on AFRRRI's CD-ROM network for literature searches.

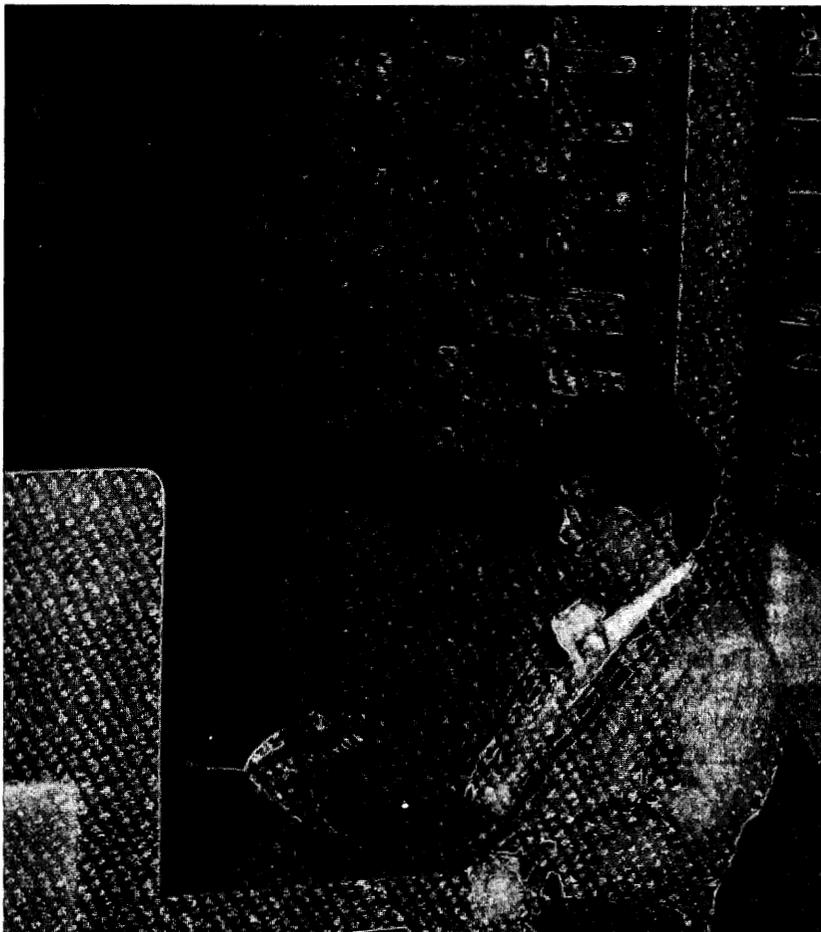
video tapes). The library maintains subscriptions to 200 scientific and professional journals and other serials, including five on compact disks with read-only memory (CD-ROM) and one on computer diskette.

In addition, the library offers bibliographic literature searches of on-line data bases, a reference service, and an interlibrary loan service. The loan service allows AFRRRI researchers access to published materials locally through the Interlibrary Loan Users Association and nationwide through the Online Computer Library Center (OCLC).

The library makes available a computer-generated monthly acquisitions list, a computer-generated bibliography of all cataloged audiovisual materials sorted according to media, and an automated data-base maintenance program.

Patrons can access an on-line catalog as well as the five CD-ROM data bases (Computer Select, DoD Hazardous Materials Information System, Medline Express, Meyler's Side Effects of Drugs, Physicians' Desk Reference). The Current Contents: Life Sciences data base is available to users on computer diskette, and access is available to the on-line catalog of the Uniformed Services University of the Health Sciences Library.

In response to the Department of Defense (DoD) initiative to re-



Dr. Alexandra Miller refers to one of the many scientific journals in AFRRRI's journal collection.

duce or eliminate duplication of effort in DoD research activities, the library subscribes to the Automatic Document Distribution (ADD) and the Current Awareness Bibliography (CAB) services available from the Defense Technical Information Center (DTIC), which is a major source for DoD and government contract technical and scientific reports. These bi-weekly services provide the li-

brary with microfiche copies (through ADD) and a paper bibliography (through CAB) of newly acquired DTIC technical reports that match the library's established subject profile.

The library is named for U.S. Navy Commander William H. Chapman, a member of the Medical Service Corps and one of the founders of AFRRRI.

Computer system enhances collection, analysis, dissemination of scientific data

A central computer system and a local area network (LAN) provide shared automated resources used to collect and analyze information, to access scientific data bases, and to produce manuscripts and posters that disseminate AFRRRI findings to audiences worldwide. Figure 1 depicts the shared computer facility.

computers with related disk drives, dial-in modems, printers, and a port switcher. Scientists use the VAXes to run RS/1 to analyze data and create graphs. The system also makes available MASS11, a powerful word processing program, and the Oracle system, which manages more than a dozen support and scientific data bases.

The central computer system has three clustered VAX mini-

The VAX 6310 has an 8-mm tape drive, which is used to per-

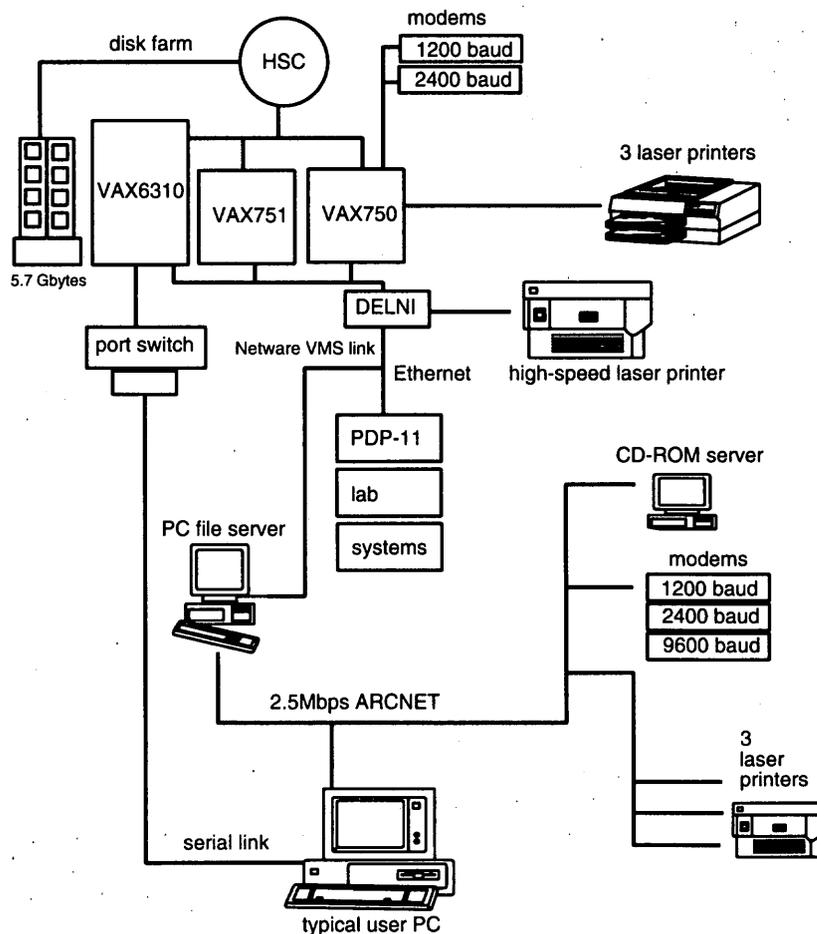


Fig. 1. AFRRRI's central computer system.

Computer and Electronics Department Head

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Ingrid M. Jackson

Capt, USAF

Hardware Division Chief

Eugene J. Herbert

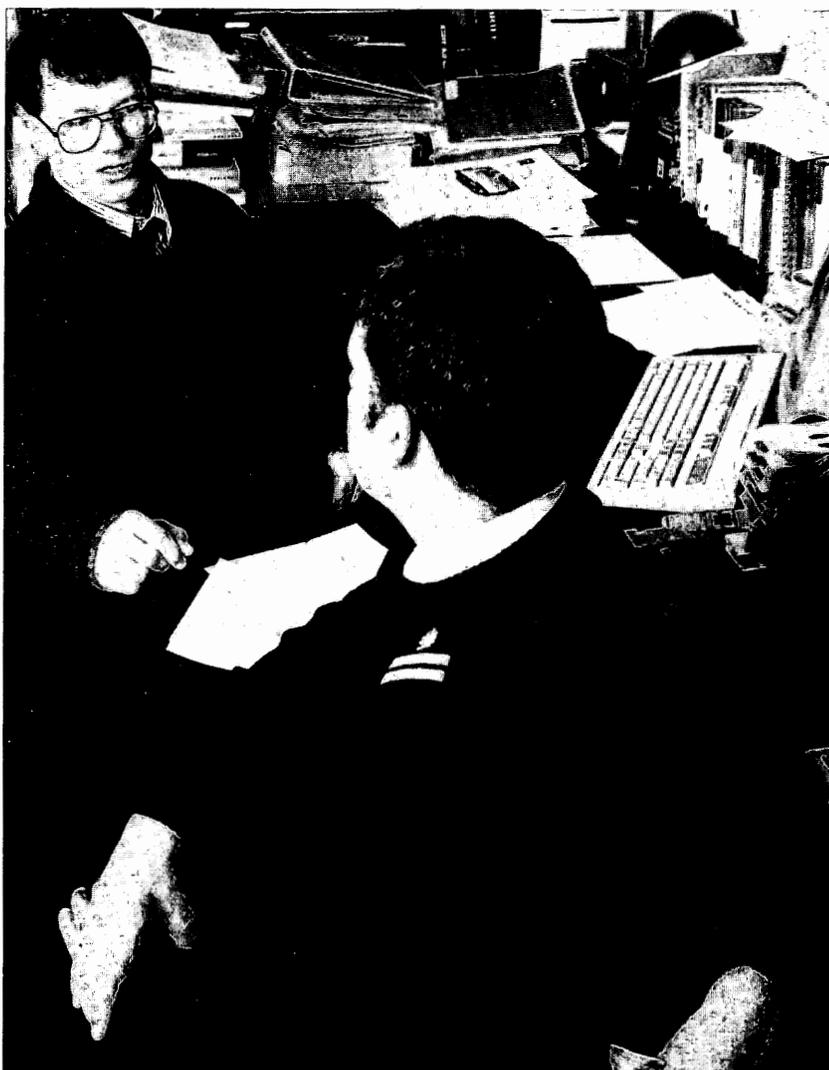
Electronics Engineers

John Chezik

Hung Phan

Electronics Technician

Gary Robey



Computer Specialist Thomas A. Lively and LT Luis Benevides, USN, discuss a proposed data acquisition program.

form unattended backup of the systems. Each 8-mm tape holds up to 2.4 gigabytes of data (equal to more than 2 billion text characters).

The Netware-VMS applications software package allows personal computer (PC) users to quickly move data between the systems, VAX users to work independent of the central computer system, and LAN users to communicate with VAX-based laser printers.

The LAN consists of two file servers, some 200 PCs linked via ARCNET, and two dial-out modems. The network lets users access CD-ROM (compact disc with read-only memory) data bases. Its electronic mail allows the exchange of information within AFRRI, and its WordPerfect software provides a widely used format for sharing information outside the organization.

Stand-alone computers, including PCs and PDP-11 mini-

computers, are used in laboratories for the collection and preliminary analysis of experimental data.

In fiscal year 1992, we continued the process of migrating multiuser computing systems to a distributed computing environment. We reconfigured a file server into an Oracle data-base server and attached it to the existing local area network (LAN). We configured personal computer (PC) workstations throughout the institute into Oracle client stations. These workstations replace dumb terminal linkages to the VAX cluster that runs Oracle. The VAX-based Oracle data-base system will be phased out in fiscal year 1993.

In addition we established a DNA/AFRRI off-site storage agreement to safeguard AFRRI computer data. A new uninterruptible power supply (UPS) was installed, tested, and put into service to increase the reliability of both the LAN and the VAX multiuser computer systems. UPS allows AFRRI's computer systems to continue to operate through short brownouts and protects against power surges that can occur during electrical storms.

Electronics engineers developed a filter wheel, a microscope system, and a light intensity controller system for the Radiation Biophysics Department; maintained and upgraded scientific apparatus throughout AFRRI, including acoustic water maze hardware and electronics for the Behavioral Sciences Department (BHS); upgraded the visual discrimination system for BHS; and designed an upgraded LAN configuration for the future installation of an Ethernet LAN.

Programmers transferred 14 Oracle data-base applications from the VAX to the LAN server; delivered acoustical water maze control software to BHS; wrote LAN-based utilities such as the program that simplifies electronic mail printing in the DOS environment; wrote software that transforms raw data files produced by an Omnitech Electronics Diet-Scan Analyzer into RS/1 loadable data sets for BHS; supported researchers by providing expert RS/1 program support such as an RPL program set to statistically analyze and produce presentation quality graphs from the analyzer data.



Capt Ingrid Jackson, USAF, assists TSgt Cheryl Adams, USAF, in the use of a word processing program on AFRRRI's local area network.

Extramural Activities

Outside research

William F. Blakely, Ph.D.

Effect of cell cycle on fission neutron-induced cell transformation. University of California at Irvine (in collaboration with L.J. Redpath, Ph.D., of the university)

Effect of radiation quality on radiation energy deposition events. Naval Research Laboratory, Bethesda, Md. (in collaboration with M.A. Xapsos, Ph.D., of the laboratory)

Effect of radiation quality on the induction of chromosome damage. Lawrence Berkeley Laboratory, Berkeley, Calif. (in collaboration with E.A. Blakely, Ph.D., of the university)

Effect of radioprotectors on fission neutron-induced cell transformation. University of Maryland School of Medicine, Baltimore, Md. (in collaboration with Elizabeth Balcer-Kubiczek, Ph.D., and George Harrison, Ph.D., both of the university)

Elsa Chock, Ph.D.

Mechanism of signal transduction in inflammatory cells. National Heart, Lung, and Blood Institute and National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md.

Thomas B. Elliott, Ph.D.

Temporal appearance of genetic expression of cytokines following treatment of irradiated mice with biological response modifiers. Uniformed Services University of the Health Sciences, Bethesda, Md., and University of Colorado Health Sciences Center, Denver, Colo. (in collaboration with William C. Gause, Ph.D., Uniformed Services University of the Health Sciences and Verlyn M. Peterson, M.D., University of Colorado Health Sciences Center)

Elaine K. Gallin, Ph.D.

Cloning of leukocyte potassium channels (in collaboration with Steven Wieland, Ph.D., Hahnemann Medical College, Philadelphia, Pa., and Philip Murphy, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.)

Pamela J. Gunter-Smith, Ph.D.

Radiation protection by prostaglandins: Mechanisms and applications. Department of Veterans Affairs/Department of Defense Merit Review Program (in collaboration with Wayne R. Hanson, Ph.D., Loyola Hines Medical Center, Hines, Ill.), Co-Principal Investigator

John F. Kalinich, Ph.D.

Effect of radiation on membrane structure and function (in collaboration with Leopold May, Ph.D., Chemistry Department, The Catholic University, Washington, D.C.)

K. Sree Kumar, Ph.D.

Interaction of nitric oxide with superoxide dismutase (in collaboration with Larry Keefer, Ph.D., National Institutes of Health, Bethesda, Md.)

Michael R. Landauer, Ph.D.

Radiation by prostaglandins: Mechanisms and applications. Department of Veterans Affairs/Department of Defense Merit Review Program (in collaboration with Wayne R. Hanson, Ph.D., Loyola Hines Medical Center, Hines, Ill.), Co-Principal Investigator, 1991-1994

R. Joel Lowy, Ph.D.

Use of video imaging to examine the fusion of viral proteins and lipids with cell membranes (in

collaboration with Robert Blumenthal, Ph.D., National Cancer Institute, National Institutes of Health, Bethesda, Md.)

Kenneth F. McCarthy, Ph.D.

Design Study. National Aeronautics and Space Administration (NASA), Houston, Texas, Co-Principal Investigator

FeFrag Study. NASA, Point of Contact

Proton Study. NASA, Principal Investigator

David E. McClain, Ph.D.

Effect of radiation on membrane structure and function (in collaboration with Leopold May, Ph.D., Chemistry Department, The Catholic University, Washington, D.C.)

Mechanisms of stress protein induction (in collaboration with Juliann G. Kiang, Ph.D., Walter Reed Army Institute of Research, Division of Medicine, Washington, D.C.)

Alexandra C. Miller, Ph.D.

Amplification of gene copy number (in collaboration with Nancy Dorsey, Ph.D., Perkin-Elmer Cetus, New Haven, Conn.)

Gene expression and calcium channels (in collaboration with Leslie McKinney Leonard, Ph.D., AFRRJ)

Gene expression and glutathione depleting agents (in collaboration with Kenneth Douglas, Ph.D., Department of Pharmacy, University of Manchester, Manchester, United Kingdom)

Gene expression and radiation resistance (in collaboration with Dvorit Samid, Ph.D., Clinical Pharmacology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.)

Lawrence S. Myers, Jr., Ph.D.

Energy and charge migration in irradiated DNA. Pacific Northwest Laboratories, Richland, Wash.

Myra L. Patchen, Ph.D.

Outside collaborative research was carried out with several biotechnology companies and

focused on evaluating the ability of newly developed cytokines to regulate and accelerate hemopoietic regeneration following radiation-induced hemopoietic aplasia (in collaboration with Sharon Aukerman, Ph.D., Cetus Corporation, Emeryville, Calif.; Lawrence Souza, Ph.D., AMGen Corporation, Thousand Oaks, Calif.; Fritz Seiler, Ph.D., Behringwerke, Marburg, Germany; Douglas Williams, Ph.D., Immunex Corporation, Seattle, Wash.; Ronald Brown, Ph.D., Quality Biologicals, Rockville, Md.; and Steven Wolpe, Ph.D., Genetics Institute, Boston, Mass.)

Terry C. Pellmar, Ph.D.

Effects of ionizing radiation on neurons in culture (in collaboration with Marion Wienrich, Ph.D., Battelle Europe, Frankfurt, Germany)

Neurotoxicity of nitroxides (in collaboration with James B. Mitchell, Ph.D., and Stephen Hahn, M.D., National Cancer Institute, National Institutes of Health, Bethesda, Md.)

Radiation and free radical effects on glial cell morphology (in collaboration with Adrienne Salm, Ph.D., University of West Virginia, Morgantown, W.Va.)

Joseph F. Weiss, Ph.D.

Radiation protection by prostaglandins: Mechanisms and applications. Department of Veterans Affairs/Department of Defense Merit Review Program (in collaboration with Wayne R. Hanson, Ph.D., Loyola Hines Medical Center, Hines, Ill.), Co-Principal Investigator

June M. Whaun, M.D.

COL, MC, USA

Mechanisms of mustard compound toxicity, especially the role of free radicals in initiating and propagating tissue damage after exposure. Walter Reed Army Institute of Research, Division of Biochemistry, Washington, D.C.

Mark H. Whitnall, Ph.D.

Effects of immune system hormones on the hypothalamus and anterior pituitary (in collaboration with Edward H. Mougey, M.S., Walter Reed Army Institute of Research, Washington, D.C.)

Expression of corticotropin-releasing hormone and vasopressin in hypothalamic neurosecretory cells during chronic inflammation (in collaboration with Stafford L. Lightman, M.D., and Michael S. Harbuz, Ph.D., Charing Cross and Westminster Medical School, London, England)

Expression of corticotropin-releasing hormone and vasopressin in the hypothalamus of mice with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (in collaboration with Ilan Irony, M.D., and Gordon Cutler, M.D., National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, Md.)

Localization of steroid sulfotransferases in the adrenal cortex (in collaboration with Charles A. Strott, M.D., Young C. Lee, Ph.D., and William J. Driscoll, Ph.D., NICHD, NIH)

The effects of central adrenergic agonists on the corticotropin-releasing hormone neurosecretory system (in collaboration with Greti Aguilera, M.D., and Alex Kiss, Ph.D., NICHD, NIH)

Awards

Mildred A. Donlon, Ph.D.

Defense Nuclear Agency Exceptional Civilian Service Medal

John F. Kalinich, Ph.D.

Defense Nuclear Agency Award for Outstanding Technical Achievement

Gregory L. King, Ph.D.

DNA Administrative Excellence Award

Marcus L. Moore

Defense Nuclear Agency Meritorious Civilian Service Award

Myra L. Patchen, Ph.D.

Selected for "Who's Who Worldwide"

June M. Whaun, M.D.

COL, MC, USA

Selected for "Who's Who in Frontier Science and Technology"

Appointed/elected positions

American Academy of Microbiology, Elected Fellow

Ruth Neta, Ph.D.

American Heart Association, Peer Review Committee

Terry C. Pellmar, Ph.D.

American Physiological Society, Member, Porter Development Committee

Pamela J. Gunter-Smith, Ph.D.

Association for Gnotobiotics, President-Elect and Board of Governors

G. David Ledney, Ph.D.

Association of Government Toxicologists, Chairman, Membership Committee

Michael R. Landauer, Ph.D.

Biophysical Society: Council Member, Chair of Public Policy Committee, Representative to Federation of American Societies for Experimental Biology Consensus Conference, and Representative to National Institutes of Health Strategic Plan Meeting in San Antonio, Texas

Elaine K. Gallin, Ph.D.

Biotherapy Journal, Editorial Board Member

Ruth Neta, Ph.D.

Department of Defense (DoD) RADIAC Working Group Executive Committee

Schleurious L. Gaiter, M.S.
LT, MSC, USN

Federation of American Societies for Experimental Biology, Visiting Scientist for Minority Institutions

Pamela J. Gunter-Smith, Ph.D.

International Conference, New Vistas on Mechanisms and Control of Emesis; Satellite Symposium: 10th Annual Meeting for European Neuroscience, Organizing Committee Member

Gregory L. King, Ph.D.

International Cytokine Workshops, Member, Program Committee

Ruth Neta, Ph.D.

International Journal of Radiation Biology, Editorial Board

Joseph F. Weiss, Ph.D.

International Program Committee, 3rd International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury

Joseph F. Weiss, Ph.D.

Journal of Immunology, Editorial Board Member

Ruth Neta, Ph.D.

Laboratory Demonstration Program

Robert L. Bumgarner, M.D.
CAPT, MC, USN
Defense Nuclear Agency (DNA) Representative, Management Subpanel

Mildred A. Donlon, Ph.D., Chairman, Legislative and Implementation Subpanels; Alternate DNA Representative, Management Subpanel

Elaine K. Gallin, Ph.D., Alternate, Legislative Subpanel

Elizabeth P. Linkins, Alternate, Personnel Subpanel

Nicholas W. Manderfield
Col, USAF, MSC
Alternate, Facilities Subpanel

Harvey G. Soefer
LTC, MS, USA
DNA Representative, Procurement Subpanel

Michael R. Ward, B.A., DNA Representative, Personnel Subpanel

Lymphokine and Cytokine Research Journal, Editorial Board Member

Ruth Neta, Ph.D.

National Board of Medical Examiners, Board Member

Pamela J. Gunter-Smith, Ph.D.

NATO Advanced Study Institute on Biological Effects and Physics of Solar and Galactic Cosmic Radiation, Director

Charles E. Swenberg, Ph.D.

NATO Panel 8, Research Study Group 23, Assessment, Prophylaxis, and Treatment of Ionizing Radiation; Delegate, and Chairman, Subgroup for Prophylaxis and Treatment of Acute Effects of Ionizing Radiation

George N. Catravas, Ph.D., D.S.C., Principal U.S. Delegate

Thomas J. MacVittie, Ph.D.

Neurotoxicology, Editorial Board Member

Paul C. Mele, Ph.D.

Oxygen Club of Greater Washington

David R. Livengood, Ph.D., Council of Past Presidents

Terry C. Pellmar, Ph.D., Secretary

Joseph F. Weiss, Ph.D., Treasurer

Oxygen Free Radicals in Brain Damage Program, External Advisory Committee, R. L. Smith Research Center, University of Kansas Medical Center

Terry C. Pellmar, Ph.D.

Society for Neuroscience, Co-Sponsor, Club Emesis

Gregory L. King, Ph.D.

Society for Neuroscience, Councilor, Potomac Chapter

Leslie McKinney Leonard, Ph.D.

Society of Air Force Physicians, Board of Governors

Alex Limanni, M.D.
Lt Col, USAF, MC

Ubiquity Science Seminar Series, Hampton University, Hampton, Va., Coordinator

Margaret Colden-Stanfield, Ph.D.

Undersea and Hyperbaric Medical Society, Executive Committee Member at Large

Robert L. Bumgarner, M.D.
CAPT, MC, USN

U.S. Office of Personnel Management 1991-1992 Executive Potential Program, Cluster Group Advisor

Mildred A. Donlon, Ph.D.

Referees for grants

Arthritis Foundation, Canada

Daniel Goldman, Ph.D.

Department of Energy

Thomas J. MacVittie, Ph.D.
Ruth Neta, Ph.D.

Department of the Navy

Paul C. Mele, Ph.D.
Peter J. Winsauer, Ph.D.

Department of Veterans Affairs Merit Review Board

Leslie McKinney Leonard, Ph.D.
Ruth Neta, Ph.D.
Myra L. Patchen, Ph.D.
Terry C. Pellmar, Ph.D.

National Cancer Institute

Doris Browne, M.D.
LTC, MC, USA

National Institutes of Health

Elaine K. Gallin, Ph.D.
Pamela J. Gunter-Smith, Ph.D.
Gregory L. King, Ph.D.
R. Joel Lowy, Ph.D.

National Institutes of Health, Division of Research Grants, Radiation Study Section

Myra L. Patchen, Ph.D.

National Institutes of Health, Study Section on Respiratory and Applied Physiology

Ruth Neta, Ph.D.

National Science Foundation

Elaine K. Gallin, Ph.D.
R. Joel Lowy, Ph.D.
Ruth Neta, Ph.D.
Mark H. Whitnall, Ph.D.

Naval Medical Research and Development Command, National Naval Medical Center

Alexandra C. Miller, Ph.D.

Referees for journals

Age and Ageing

Ruth Neta, Ph.D.

Agents and Actions

Alexandra C. Miller, Ph.D.

American Association for Laboratory Animal Science

Robert H. Weichbrod, M.B.A., LATG

American Journal of Physiology

Elaine K. Gallin, Ph.D.

Pamela J. Gunter-Smith, Ph.D.

Annals of Internal Medicine

Myra L. Patchen, Ph.D.

Applied Occupational and Environmental Hygiene

William F. Blakely, Ph.D.

Biochimica et Biophysica Acta

Elaine K. Gallin, Ph.D.

Ruth Neta, Ph.D.

Biophysical Journal

R. Joel Lowy, Ph.D.

Blood

Ruth Neta, Ph.D.

Brain Research

Terry C. Pellmar, Ph.D.

Cancer Research

Ruth Neta, Ph.D.

Myra L. Patchen, Ph.D.

Cellular Immunology

Ruth Neta, Ph.D.

Cytokine

Ruth Neta, Ph.D.

Endocrinology

Mark H. Whitnall, Ph.D.

Epithelial Cell Biology

Roy M. Vigneulle, Ph.D.

Experimental Hematology

Elaine K. Gallin, Ph.D.

Thomas J. MacVittie, Ph.D.

Kenneth F. McCarthy, Ph.D.

Ruth Neta, Ph.D.

Myra L. Patchen, Ph.D.

Free Radicals in Biology and Medicine

Terry C. Pellmar, Ph.D.

Immunology and Infectious Diseases

Ruth Neta, Ph.D.

Immunopharmacology

Ruth Neta, Ph.D.

International Journal of Cell Cloning

Thomas J. MacVittie, Ph.D.

International Journal of Immunopharmacology

Myra L. Patchen, Ph.D.

International Journal of Radiation Biology

William F. Blakely, Ph.D.

Gregory L. King, Ph.D.

Thomas J. MacVittie, Ph.D.

Kenneth F. McCarthy, Ph.D.

Ruth Neta, Ph.D.

Myra L. Patchen, Ph.D.

Roy M. Vigneulle, Ph.D.

Joseph F. Weiss, Ph.D.

Journal of Biological Response Modifiers

Myra L. Patchen, Ph.D.

Journal of Cellular Physiology

Kenneth F. McCarthy, Ph.D.

Journal of Clinical Investigation

Elaine K. Gallin, Ph.D.

Ruth Neta, Ph.D.

Journal of Experimental Medicine

Elaine K. Gallin, Ph.D.

Journal of Histochemistry and Cytochemistry

Elsa Chock, Ph.D.

Journal of Immunology

Elaine K. Gallin, Ph.D.

Daniel Goldman, Ph.D.

Myra L. Patchen, Ph.D.

Journal of Leukocyte Biology

Ruth Neta, Ph.D.

Journal of Membrane Biology

Elaine K. Gallin, Ph.D.

Leslie McKinney Leonard, Ph.D.

Journal of Neurochemistry

Leslie McKinney Leonard, Ph.D.

Journal of Pharmacology and Experimental Therapeutics

Daniel Goldman, Ph.D.

Gregory L. King, Ph.D.

Myra L. Patchen, Ph.D.

Journal of the National Cancer Institute

Ruth Neta, Ph.D.

Laboratory Animal Management Association

Robert H. Weichbrod, M.B.A., LATG

Laboratory Animals

Robert H. Weichbrod, M.B.A., LATG

Laboratory Investigation

Ruth Neta, Ph.D.

Neuroendocrinology

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Neuropharmacology

Joseph F. Weiss, Ph.D.

Neuroscience

Terry C. Pellmar, Ph.D.

Neurotoxicology

Paul C. Mele, Ph.D.

Pharmacy and Toxicology

Martha L. Hale, Ph.D.

Physiology and Behavior

Michael R. Landauer, Ph.D.

Proceedings of the National Academy of Sciences of the U.S.A.

Ruth Neta, Ph.D.

Proceedings of the Society for Experimental Biology and Medicine

Ruth Neta, Ph.D.

Prostaglandins

Daniel Goldman, Ph.D.

Radiation and Environmental Biophysics

William F. Blakely, Ph.D.

Radiation Protection DosimetryEric E. Kearsley, Ph.D.
CDR, MSC, USN**Radiation Research**

William F. Blakely, Ph.D.

George N. Catravas, Ph.D., D.Sc.

Thomas J. MacVittie, Ph.D.

Kenneth F. McCarthy, Ph.D.

David E. McClain, Ph.D.

Ruth Neta, Ph.D.

Myra L. Patchen, Ph.D.

Terry C. Pellmar, Ph.D.

Roy M. Vigneulle, Ph.D.

Joseph F. Weiss, Ph.D.

Research Communications in Chemical Pathology
and Pharmacology

George N. Catravas, Ph.D., D.Sc.

Science

Elaine K. Gallin, Ph.D.

Ruth Neta, Ph.D.

Terry C. Pellmar, Ph.D.

Teaching

Advanced Cardiac Life Support Instructor

Robert S. Perlstein, M.D.
Col, USAF, MC

Advanced Trauma Life Support Instructor

Glen I. Reeves, M.D.
Col, USAF, MC, SFS

AFRRI, Medical Effects of Nuclear Weapons
Course

William Baker, D.V.M.
LTC, VC, USA

Victor Bogo, M.S.

Itzhak Brook, M.D.
CDR, MC, USN

Doris Browne, M.D.
LTC, MC, USA

Robert L. Bumgarner, M.D.
CAPT, MC, USN

Edward P. Clark, Ph.D.

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LT, MSC, USNR

Daniel Collins, Ph.D.
Lt Col, USAF

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LTJG, MSC, USNR

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CAPT, MSC, USN

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Alan H. Harris, Ph.D.

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Lt Col, USAF, MC

Thomas J. MacVittie, Ph.D.

Alexandra C. Miller, Ph.D.

Harold E. Modrow, Ph.D.
MAJ, MS, USA

Dominic L. Palazzolo, Ph.D.
CPT, MS, USA

Myra L. Patchen, Ph.D.

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Col, USAF, MC

Glenn I. Reeves, M.D.
Col, USAF, MC

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Adjunct Associate Professor

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Catholic University, Center for Advanced Training
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Harford Community College, Chemistry Department, Bel Air, Md.

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Laurel Elementary School, Laurel, Md., Teaching Assistant

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LT, MSC, USNR

Malcolm Grow USAF Medical Center, Andrews Air Force Base, Md., Consultant in Endocrinology and Internal Medicine

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Col, USAF, MC

Montgomery College, Physical Education Department, Rockville, Md., Volunteer Faculty Member

Sara C. Gilman, Ph.D.
LCDR, MSC, USNR

National Naval Medical Center, Bethesda, Md., Attending Physician in Endocrinology and Internal Medicine

Robert S. Perlstein, M.D.
Col, USAF, MC

National Naval Medical Center, Bethesda, Md., Nuclear Medicine Course

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National Naval Medical Center, Bethesda, Md., Staff Pathologist

Robert L. Bumgarner, M.D.
CAPT, MC, USN

Oak Ridge Institute for Science and Education, Radiation Emergency Assistance Center/Training Site, Oak Ridge, Tenn., Guest Faculty

Doris Browne, M.D.
LTC, MC, USA

Thomas J. MacVittie, Ph.D.

Spelman College Biophysics Lecture Series, Atlanta, Ga.

Pamela J. Gunter-Smith, Ph.D.

Thomas W. Pyle Middle School, Bethesda, Md., Gifted and Talented Program

Mildred A. Donlon, Ph.D.

Uniformed Services University of the Health Sciences, Bethesda, Md., Assistant Professor of Internal Medicine

Alex Limanni, M.D.
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Pamela J. Gunter-Smith, Ph.D., Adjunct Assistant Professor

Thomas J. MacVittie, Ph.D., Lecturer

Uniformed Services University of the Health Sciences, Women and Minorities in Medicine Program, Bethesda, Md.

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University of Maryland School of Medicine,
Department of Radiation Oncology, Baltimore, Md.

William F. Blakely, Ph.D.

University of Pisa School of Medicine, Pisa, Italy,
Visiting Professor

Joseph F. Weiss, Ph.D.

Walter Reed Army Medical Center, Department of
Endocrinology and Internal Medicine, Washington,
D.C., Attending Physician

Robert S. Perlstein, M.D.
Col, USAF, MC

Walter Reed Army Medical Center, Department of
Medicine and Rheumatology, Washington, D.C.,
Attending Physician

Alex Limanni, M.D.
Lt Col, USAF, MC

Sudent sponsorship

AFRRI Cooperative Student Program, Mentor for
C. A. Lane

Mark H. Whitnall, Ph.D.

AFRRI Fellow Sponsor

G. David Ledney, Ph.D.

AFRRI/University of Maryland Cooperative
Student Program, Mentor for J. P. Glover

Gregory L. King, Ph.D.

Bethesda Chevy Chase High School, Bethesda,
Md., Sponsor for Michael Chen from January to
August 1992

Leslie McKinney Leonard, Ph.D.

Brown University, Providence, R.I., Sponsor for
Leela E. Noronha from July to September 1992

William F. Blakely, Ph.D.

College of William and Mary, Williamsburg, Va.,
Sponsor for Christian Wilson from June to August
1992

Eric G. Daxon, Ph.D.
LTC, MS, USA

Department of Defense, Science and Engineering
Apprenticeship Program for High School Students,
Mentors

Margaret Colden-Stanfield, Ph.D.

Thomas B. Elliott, Ph.D.

Elaine K. Gallin, Ph.D.

Gregory L. King, Ph.D., AFRRI Coordinator

Leslie McKinney Leonard, Ph.D.

Roy M. Vigneulle, Ph.D.

Jackie L. Williams, Ph.D.
MAJ, MS, USA

Gaithersburg High School, Gaithersburg, Md.,
Sponsor for Thomas Dugan, summer 1992

Sara C. Gilman, Ph.D.
LCDR, MSC, USNR

Georgetown University, Washington, D.C.,
Sponsor for Neal Barss from January to May 1992

Henry M. Gerstenberg, M.S.

Metropolitan Washington, D.C., area and Gallaudet
College, Sponsor for high school and college
students

G. David Ledney, Ph.D.

National Science Foundation Fellow Sponsor

G. David Ledney, Ph.D.

North Carolina Medical School, Sponsor for
Michael G. Summer from June to July 1992

William F. Blakely, Ph.D.

Thomas Jefferson High School for Science and
Technology, Alexandria, Va., Mentorship Program,
Mentors

Thomas B. Elliott, Ph.D.

Elaine K. Gallin, Ph.D.
Roy M. Vigneulle, Ph.D.

Uniformed Services University of the Health Sciences, Department of Microbiology, Bethesda, Md., Doctoral Candidate Sponsor and Research Advisor

G. David Ledney, Ph.D.

University of Maryland, Experiential Learning Program, College Park, Md., Sponsor for Consuella R. Matthews

David E. McClain, Ph.D.

U.S. Naval Academy, Annapolis, Md., Sponsor for Mark Peters from May to June 1992

Paul T. Kaiser, Ph.D.
LT, MSC, USN

Walter Reed Army Institute of Research, Washington, D.C., Mentor to college-aged technical staff members

June M. Whaun, M.D.
COL, MC, USA

Wootton High School, Potomac, Md., Sponsor for Cicily Daniels, summer 1992

Sara C. Gilman, Ph.D.
LCDR, MSC, USNR

Wootton High School, Potomac, Md., Sponsor for Neerav Mehta

Robert S. Perlstein, M.D.
Col, USAF, MC

Judging/examining/evaluating

Consultant to the Office of The Surgeon General of the Army for selection of medical personnel for Army Reserve appointments and for participation in military scholarship programs

June M. Whaun, M.D.
COL, MC, USA

George Washington University, Washington, D.C., Thesis Committee, Department of Biology

Elaine K. Gallin, Ph.D.

Montgomery County High School Science Fair

Itzhak Brook, M.D.
CDR, MC, USN

Myra L. Patchen, Ph.D.

Joseph F. Weiss, Ph.D.

June M. Whaun, M.D.
COL, MC, USA

Jackie L. Williams, Ph.D.
MAJ, MS, USA

Thomas Jefferson High School for Science and Technology Science Fair, Alexandria, Va.

Thomas B. Elliott, Ph.D.

U.S. Army NCO Selection Board

Doris Browne, M.D.
LTC, MC, USA

Presentations

(at AFRRRI unless otherwise noted)

E. John Ainsworth, Ph.D.

Overview of the use of animals in radiation research, historical, and at AFRRRI. Committee on Interagency Radiation Research and Policy Coordination Meeting. May 1992

Ramesh Bhatt, Ph.D.

The LD_{50/30} of fast neutrons in the dog. October 1991

William F. Blakely, Ph.D.

Development of automated micronucleus assay: Automated PCC-assay. Workshop of the Research Study Group 23 on the Assessment, Prophylaxis, and Treatment in Nuclear Environments, The Hague, Netherlands. May 1992

Doris Browne, M.D.

LTC, MC, USA

Health effects of radiation exposure and radionuclide dispersal (sponsored by the Department of Energy). Nuclear Awareness for the 1990's and Beyond Seminar, Hertford, N.C. August 1992

George N. Catravas, Ph.D., D.Sc.

Review of medical/biological damage, biological dosimeters, and radioprotection. Workshop of the Research Study Group 23 on the Assessment, Prophylaxis, and Treatment in Nuclear Environments, The Hague, Netherlands. May 1992

Warren Chen, Ph.D.

Work in progress: Studies using microdialysis. May 1992

Margaret Colden-Stanfield, Ph.D.

HL60 cells seeking influenza virus-infected endothelium: The final episode. May 1992

Eric G. Daxon, Ph.D.

LTC, MSC, USA

ICRP models for internal dosimetry (first seminar in a series on internal contamination). January 1992

Thomas B. Elliott, Ph.D.

Therapies for bacterial sepsis in combined injured mice. January 1992

Elaine K. Gallin, Ph.D.

Work in progress: Studies using the perforated patch technique. June 1992

Pamela J. Gunter-Smith, Ph.D.

Gastrointestinal ion transport: A progress report. June 1992

Susan Judge, Ph.D.

Work in progress: Studies using the perforated patch technique. June 1992

Paul T. Kaiser, Ph.D.

LT, MSC, USNR

AFRRI officer development guide: Tri-service military and professional training and career development. August 1992

John F. Kalinich, Ph.D.

An in vitro nuclear transport system. Cell and Molecular Biology Affinity Group. January 1992

Radiation effects on nuclear transport. Radiation Biochemistry Department Peer Review. December 1991

Gregory L. King, Ph.D.

Ethical considerations and role of animal care and use committees. Committee on Interagency Radiation Research and Policy Coordination Meeting. May 1992

Possible potentiation of the emetic response to oral S(-)zacopride in the ferret by various receptor ligands. International Conference on New Vistas on Mechanisms and Control of Emesis, Marseille, France; 10th Annual Meeting for European Neuroscience, Munich, Germany. September 1992

Kenneth Kirschner, M.S.

LT, MSC, USN

Serum cytokine changes in primates. April 1992

R. Joel Lowy, Ph.D.

Influenza virus-cell interactions observed using fluorescent video microscopy. October 1991

Thomas J. MacVittie, Ph.D.

Therapeutic efficacy of combined cytokine protocols (IL-3 plus GM-CSF) in irradiated monkeys. Workshop of the Research Study Group 23 on the Assessment, Prophylaxis, and Treatment in Nuclear Environments, The Hague, Netherlands. May 1992

Milan Makale, Ph.D.

Role of the autonomic nervous system in induced emesis in the ferret. July 1992

David E. McClain, Ph.D.

Calcium metabolism in irradiated MOLT-4 nuclei. Cell and Molecular Biology Affinity Group. March 1992

Radiation effects on nuclear transport. Radiation Biochemistry Department Peer Review. December 1991

Albert H. McCullen, D.V.M.
LTC, VC, USA

Role of VSD in support of AFRRRI research. Committee on Interagency Radiation Research and Policy Coordination Meeting. May 1992

Kent M. McLean, Ph.D.
Lt Col, USAF, BSC

Increased radiation resistance in *E. coli* JM83 is associated with a chromosomal rearrangement. January 1992

Alexandra C. Miller, Ph.D.

Modulation of *ras* gene transcription by glutathione depleting agents. Oncogene Meeting of the Foundation for Cancer Research, Frederick, Md. June 1992

Relationship of *ras* expression and radio-resistance. Institute of Electrical and Electronics Engineers, Engineers in Medicine and Biology Society Meeting, Orlando, Fla., October 1991

Harold E. Modrow, Ph.D.
MAJ, MS, USA

Diagnosis and treatment of acute radiation syndrome and combat stress reaction on the integrated battlefield. Combat and Disaster Medical Symposium, Army Nurse Corps Education, 42nd Field Hospital, Fort Knox, Ky. May 1992

Elizabeth Montcalm-Mazzilli, Ph.D.
LT, MSC, USNR

Mechanisms of induction of gastric mucosal eicosanoids in response to noxious stimuli. March 1992

Terry C. Pellmar, Ph.D.

Dose and dose-rate effects of ionizing radiation on hippocampal electrophysiology. June 1992

Glen I. Reeves, M.D.
Col, USAF, MC, SFS

Fractionation in clinical radiation therapy: New directions. March 1992

Health effects from the Chelyabinsk nuclear accidents. July 1992

Charles E. Swenberg, Ph.D.

Does the topology of closed supercoiled DNA affect its radiation sensitivity? NATO Advanced Study Institute on Biological Effects and Physics of Solar and Galactic Cosmic Radiation, Algarve, Portugal. October 1991

Mark Whitnall, Ph.D.

Effects of IL-1 on corticotropin-releasing hormone. December 1991

Functional studies of corticotropin-releasing hormone/arginine vasopressin. September 1992

Invited seminars

William F. Blakely, Ph.D.

Mechanisms of H₂O₂-induced DNA damage and cytotoxicity. Anatomy and Cell Biology Department, Uniformed Services University of the Health Sciences, Bethesda, Md., November 1991

Doris Browne, M.D.
LTC, MC, USA

Breast cancer in minority women. National Trends Committee, LINKS, Inc., Philadelphia, Pa., keynote speaker, April 1992

Alasdair J. Carmichael, Ph.D.

Active oxygen and nitrogen species and intestinal peristalsis. Oxygen Club of Greater Washington, Bethesda, Md., January 1992

Reactions of active oxygen and nitrogen species studied by electron paramagnetic resonance and spin trapping. 3rd International Congress on Spin Trapping and Aminoxyl Radical Chemistry, Kyoto, Japan, November 1991

Margaret Colden-Stanfield, Ph.D.

Keynote speaker, Minority Undergraduate Research Symposium, University of Texas Medical Branch, Galveston, Texas, February 1992

Patch clamp and functional studies in endothelial cells. Department of Pharmacology, University of Texas Medical Branch, Galveston, Texas, February 1992

Mildred A. Donlon, Ph.D.

Getting to the senior executive service. Department of Defense Forum, National Conference of Federally Employed Women, Cincinnati, Ohio, July 1992

Pamela J. Gunter-Smith, Ph.D.

Physiologic role of an apical membrane voltage-dependent potassium conductance in gallbladder epithelial cells. Savannah State College, Savannah, Ga., May 1992

R. Joel Lowy, Ph.D.

Dispersion of influenza virus molecular components during fusion observed using video microscopy. Workshop on Membrane Fusion, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md., April 1992

Influenza virus fusion to red blood cells using fluorescent video microscopy. Genzyme, Inc., Natick, Mass., May 1992

Alexandra C. Miller, Ph.D.

New frontiers in radiation protection: *ras* expression and radiation protection. Institute of Electrical and Electronics Engineers, Engineers in Medicine and Biology Society, Orlando, Fla., October 1991

Radiation resistance and oncogenes. Institut für Biologie IV (Mikrobiologie) - Rheinisch

Westfälische Technische Hochschule, Aachen, Germany, September 1992

Ruth Neta, Ph.D.

Cytokines in protection from radiation injury. Departments of Oncology and Immunology, Haddasah Medical School, Jerusalem, Israel, February 1992

Cytokines in protection from radiation injury. Institute for Cancer Research, Bar Ilan University Medical School, Tel Aviv, Israel, February 1992

Cytokines in protection from radiation injury. Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md., September 1992

Laboratory microbiology and immunology. National Institute of Dental Research, National Institutes of Health, Bethesda, Md., March 1992

Myra L. Patchen, Ph.D.

Therapy for radiation-induced hemopoietic injury: From bugs to cytokines. Loyola University, Chicago, Ill., July 1992

Terry C. Pellmar, Ph.D.

Free radical exposure of hippocampal neurons: Electrophysiological consequences. Neuroscience Program, Uniformed Services University of the Health Sciences, Bethesda, Md.

Free radical exposure of hippocampal neurons: Electrophysiological consequences. Physiology Department, University of Maryland, Baltimore, Md.

Glen I. Reeves, M.D.

Col, USAF, MC, SFS

Airway management. Advanced trauma life support. Uniformed Services University of the Health Sciences, Bethesda, Md., January 1992

Life span studies on the carcinogenic effects of heavy charged particles in rodents. National Aeronautics and Space Administration Contractors Meeting, Houston, Texas, April 1992

The health effects of chronic whole-body radiation exposure. 1st International Workshop on the Chelyabinsk Nuclear Accidents and Their Consequences, George Mason University, Fairfax, Va., June 1992

Linda Steel-Goodwin, Ph.D.
Capt, USAF, BSC

Active oxygen and nitrogen species and intestinal peristalsis. Oxygen Club of Greater Washington, Bethesda, Md., January 1992

Nitrogen-centered free radicals: Toxicological and beneficial aspects. Toxicology Division, Wright-Patterson Air Force Base, Dayton, Ohio, September 1992

Scientific society/organization memberships

Aerospace Medical Association

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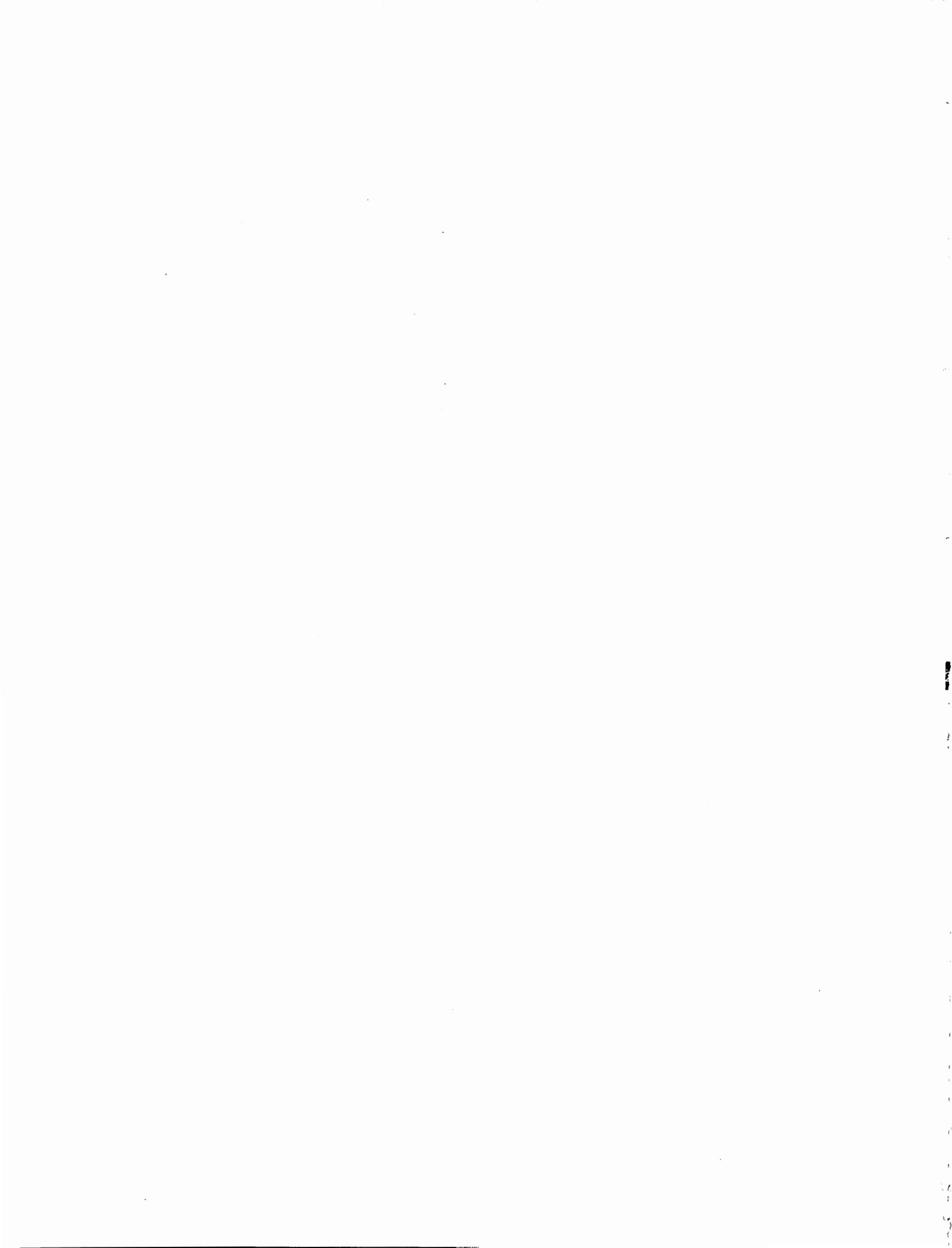
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