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AFRRI 5 YEAR PLAN

FY 83 - 87

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Armed Forces Radiobiology Research Institute
Defense Nuclear Agency

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Fiscal Years 1983 - 1987

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Defense Nuclear Agency

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INTRODUCTION

Planning for military operations in which nuclear weapons may be used requires information not currently available concerning the effects of ionizing radiation on man. Given this incomplete state of knowledge and the fact that radiation can adversely affect both the combat performance effectiveness and survivability of personnel, the military departments have identified requirements for research to define the effects of ionizing radiation on military personnel. Providing the research to meet these requirements is the primary responsibility of the Armed Forces Radiobiology Research Institute (AFRRI), which is chartered as the principal radiobiological research laboratory for the Department of Defense (DoD). AFRRI's mission is to conduct research in the field of radiobiology and related matters that are essential to the operational and medical support of DoD and the military departments. To support that mission, AFRRI is expected to perform several related functions: (a) to conduct a comprehensive in-house radiobiology research and training program; (b) to monitor, as appropriate, any outside contracts necessary to satisfy military departments and other DoD requirements in this broad research field; and (c) to provide analysis, study, coordination, and consultation on how the biological effects of ionizing radiation may affect the ability of military personnel to accomplish their operational mission on an integrated battlefield.

Realistic fiscal constraints were considered in developing this plan in that it was recognized that future budget funding levels would have certain limits. Although this was not written specifically to conform with currently projected funding levels for the years covered by the plan, the intent was to maintain a degree of reasonableness in the amount of research expected to be conducted during those years. It should be noted that currently approved AFRRI budget and manpower authorizations may not be sufficient to accomplish all research set forth in the plan. Budget requests to be submitted for each year of the plan will have to consider the projected costs for research ultimately designated for accomplishment during that given year.

The categories into which the biomedical effects program in this plan is divided represent the broad view of the DoD total effort in biomedical effects research. Although it is recognized that AFRRI, as the principal DoD radiobiological research laboratory, should retain responsibility for conducting or monitoring the majority of the research effort in this field, it is appreciated that radiobiological expertise also resides at other Defense and civilian organizations. That expertise is considered valuable and essential to the total Defense radiobiological program. A significant principle of this plan is the recognition of the need for an integrated biomedical effects research program to ensure that results of research at the key facilities are shared and that research of one will complement research conducted at other facilities.

The AFRRI research program is designed to address requirements that meet the needs of the military departments for information on the operational and medical impact of ionizing radiation. Research for mission requirements in these two areas constitutes the major portion of the AFRRI effort. This work is supported by basic research that provides the

radiobiological technology base for the efforts in operational and medical areas. A significant outgrowth of this research effort is the Medical Effects of Nuclear Weapons education program that provides training to satisfy internal Institute needs, provides training in response to identified requirements of the Service medical and operational communities, and disseminates to the Services knowledge in the field of biological effects of radiation. To provide a point of integration and a rapid mechanism for analysis of relevant radiobiology information, AFRRI is undertaking the construction of a computerized Comprehensive Personnel Nuclear Weapons Effects Model designed to facilitate the analysis of operational and medical radiobiology problems and the assimilation of AFRRI research data into militarily useful information.

PRIORITY OF RESEARCH EFFORT

One of the most important aspects of any plan, and also one of the most difficult to achieve, is the matter of setting the priority in which the separate parts of the plan will be undertaken. The task of assigning priorities to the appropriate categories of the biomedical effects program was made more difficult by the fact that this research plan must satisfy the need for pertinent information as expressed by not only the separate Services but also by both the operational and the medical communities within each Service. However, with the assistance of representatives from these various communities, this divergence in requirements was addressed, and the following assignment of relative priorities was developed for the AFRRI research plan.

The underlying concept used in determining these priorities was that the highest order of priority must be given to providing scientific answers that will better enable the field commanders to accomplish their respective missions. Both the operational and medical field commanders must be provided information from biomedical research that will enhance their capabilities and improve performance of their primary missions. The requirements expressed by the operational and medical communities are actually reverse sides of a coin; they simply view the questions and use the resultant answers from different perspectives. This plan intends to bring closer those perspectives by first setting specific requirements and then addressing past research as well as that research necessary to satisfy those requirements. Although the nature of this plan results in a scientific document, the AFRRI goal is to provide information in the form and terminology appropriate for the intended recipient.

These priorities are assigned on the basis of respective levels of effort rather than on a straight numbering scheme. Assigning absolute priorities to general research areas has certain inherent dangers; using these levels of effort for setting priorities is our attempt to overcome such dangers as much as possible. Definitions of these priorities are as follows. Actual categories and their respective priorities are shown on the following page.

a. Priority A - This applies to those highest priority areas for which a significant level of effort must be devoted.

b. Priority B - This applies to those high priority areas for which a meaningful level of effort should be devoted.

c. Priority C - This applies to those areas for which a reasonable level of effort should be devoted consistent with available resources.

d. Priority D - This applies to those areas within the Technology Base that provide basic knowledge supporting other research efforts in the biomedical program. Although this is recognized as an important area, it is anticipated that resources devoted to the Technology Base research will not exceed 20% of the annual budget.

BIOMEDICAL EFFECTS RESEARCH PROGRAM

By Major and Subordinate Category

Category	Priority
NUCLEAR WEAPONS EFFECTS EDUCATION	A
DEGRADATION OF COMBAT PERFORMANCE	
Effect of Ionizing Radiation on Combat Effectiveness	A
Mechanisms of Performance Decrement and Incapacitation	A
Effect of Drugs on Combat Performance	A
Combined Effects on Combat Performance	A
PREVENTION AND TREATMENT OF RADIATION EFFECTS	
Radioprotectants: Chemical and Physical	A
Radiation-Induced Cardiovascular Dysfunction	B
Radiation-Induced Gastrointestinal Dysfunction	B
Radiation-Induced Hematopoietic Dysfunction	B
Medical and Surgical Therapy of Combined Effects	A
DOSIMETRY	
Biologic Dosimetry	C
Physical Dosimetry	C
EFFECTS OF FAST NEUTRONS	
Biomedical Effects	C
Decrement of Performance	C
TECHNOLOGY BASE	
Biochemistry	D
Experimental Hematology	D
Radiation Sciences	D
Physiology	D
Behavior Sciences	D

EXECUTIVE SUMMARY

NUCLEAR WEAPONS EFFECTS EDUCATION

The objective of the Education Program of AFRRI is to improve the operational readiness of our forces through dissemination of the latest available information on the medical aspects of nuclear weapons. A number of programs are conducted, ranging from a 3-hour symposium to a biannual 1-week course. A special 14-hour block is taught to first-year medical students at the Uniformed Services University of the Health Sciences. During the past year, increasing requests were received for mini-courses on weapons effects. AFRRI will continue to satisfy as many requests as its education budget can support.

DEGRADATION OF COMBAT PERFORMANCE

Effect of Ionizing Radiation on Combat Effectiveness

Already stated are the military requirements for information on both individual and unit performance degradation due to the behavioral and physiological effects of exposure to ionizing radiation, as a function of radiation dose, quality, dose rate, and time after exposure. To meet those requirements, several disciplines and approaches must be integrated. Since human data on radiation effects are few and since animal experiments cannot be comprehensive models of human performance, confident predictions of radiation-induced combat ineffectiveness can be derived only by applying data on the degradation of specific functions to the actual task analyses of critical military tasks. Thus, the overall approach in meeting the requirements for information on radiation-induced performance decrement will be to:

- carry out specific animal experiments to quantify radiation-produced degradation on the critical combat functions for which data do not exist,
- quantify the frequency and duration of prodromal radiation effects such as nausea and vomiting for midlethal to sublethal doses,
- combine these data with existing human data on similar radiation effects,
- establish descriptive models for radiation-induced performance decrement,
- define task analyses for critical combat tasks,
- apply the degradation data developed from the experiments to the task analyses and attempt to predict combat ineffectiveness,
- combine degradation analyses for crews in order to obtain information on unit ineffectiveness, and

- integrate this information into the development of a Comprehensive Personnel Nuclear Weapons Effects Model (CPNWEM).

Mechanisms of Performance Decrement and Incapacitation

Understanding the physiological basis for radiation-induced incapacitation and performance decrement is essential in order to provide protection against these radiation effects. It is necessary to verify the extrapolation of data from animals to man and to develop general models of radiation-produced performance decrement. Performance decrement postirradiation comprises a spectrum of responses from no effect, or malaise, to a variety of incapacitated states. To determine these mechanisms, the following factors will be assessed:

- hypotension after irradiation, changes in membrane permeability and neurotransmitter release, changes in monosynaptic reflexes, measurements of alteration in neuronal and glial interactions, and mechanisms of damage produced by chemicals that simulate radiation-produced incapacitation;

- regional cerebral blood flow (RCBF) measured by conventional invasive techniques with implanted cerebral electrodes;

- RCBF measured by non-invasive techniques using radionuclides in combination with multiple arrayed detectors, and single photon emission computed tomography (SPECT);

Effect of Drugs on Combat Performance

The integrated battlefield poses a significant hazard not only because of the potential effects of chemical, nuclear, and biological agents but also because of the self-administration of a host of drugs: radioprotectants, antiemetics, chemical antidotes, vaccines, over-the-counter drugs, alcohol, and recreational drugs. The major drugs will be evaluated for behavioral and physiological dysfunction pre- and postirradiation.

Combined Effects on Combat Performance

The interaction of nuclear weapons effects and chemical agents is of singular importance on the integrated battlefield. The effects of chemical and radiation insults will be evaluated by biochemical, electrophysiological, and radionuclide techniques. Attempts to mitigate these effects will also be studied, especially the possibility of reversible "opening" of the blood-brain barrier to chemical antidotes and radioprotectants.

TREATMENT OF RADIATION EFFECTS

Radioprotectants

The Walter Reed Army Institute of Research has devoted a tremendous amount of its research to the development of radioprotective drugs. Some of these drugs show minimum toxicity in humans, offer significant protection, and may be available for oral use within 5 years. The best

radioprotective drug developed thus far is WR2721. AFRRI has initiated research to evaluate the efficacy of this drug and different dietary adjuvants such as vitamins B_6, A, and E as possible radioprotectants. Multiple combinations of these radioprotectants will be assessed for different qualities of ionizing radiation.

Radiation-Induced Organ System Dysfunction

Rapidly proliferating tissues, such as bone marrow and gastrointestinal tract epithelium, are the most susceptible to radiation. Radiation-induced damage to these tissues leads to symptoms such as nausea, vomiting, diarrhea, anemia, bleeding, and infection. Therefore, means must be developed to (a) increase white cell production, (b) devise techniques that can rapidly separate, type, and store white cells for infusion into irradiated personnel, and (c) devise methods to prevent the sequelae of gastrointestinal injury. Bone marrow transplantation and infusion of selected peripheral blood cell populations will be established in large animal models for the rescue of lethally irradiated recipients. Reliable large-animal models for radiation injury, postirradiation sepsis, and combined injury will be established. A valid data base will be developed for the effects of these variables on the prognosis and treatment of combined radiation injuries on the nuclear battlefield. The efficacy of transplantation and transfusion procedures will also be determined in these models.

Medical and Surgical Treatment of Combined Effects

Blast and thermal energy in combination with radiation may be found in a future combat environment. Other factors, such as chemical and biological warfare agents and nonionizing radiation, are also very likely to be present. Therefore, it is important to determine the impact of these agents in combination with ionizing radiation on the overall response of personnel. Research will be conducted to:

- evaluate blood-forming tissues and the immune system in the combined-injury individual;
- determine the effectiveness of radioprotectants and other interventions in increasing survival and in treating combined-injury personnel;
- evaluate surgical procedures with noncontaminated wounds, contaminated wounds, and similar wounds after radiation.

DOSIMETRY

Biologic Dosimetry

Development of improved dosimetric techniques that can be used in combat for the triage of military personnel are of utmost importance to field commanders and medical officers. Techniques for measuring biological indicators of radiation injury in physiological fluids have been the subject of extensive research at AFRRI. These techniques will be reassessed, and a systematic analysis of selected serum and urine components from irradiated animals will be made, using the techniques of greatest promise. The

techniques developed for different qualities of radiation will then be validated, followed by automation of the developed assay techniques, for possible use in the battlefield. Electron paramagnetic resonance spectrometry has been used to detect the radiation-induced formation of free radicals in bone, teeth, and nails. A systematic study, begun at AFRRI, will continue to evaluate these non-invasive techniques as possible indicators of radiation injury. Fluorescence-activated cell sorting (FACS-II) has been shown to rapidly and reliably evaluate many physical and biochemical factors of peripheral blood cells using two lasers. It is hoped that rapid separation of stem cells will lead to useful dose-response relationships.

Physical Dosimetry

An improved dosimetric system will be developed to allow measurement of free-in-air dose, midline tissue dose, and bone marrow dose for different quantities and qualities of radiation. This system should be applicable to monoenergetic and polyenergetic neutron fields, gamma fields, and mixed neutron-gamma fields, as found in a combat environment. The reactor fields will be mapped in addition to the development of microdosimetric capability.

EFFECTS OF FAST NEUTRONS

The relative biological effectiveness (RBE) of neutrons has been a hotly contested scientific question, in large part because of the variable biological end point used in its determination. RBE varies, depending on neutron energy, dose rate, total dose, and the biologic system studied. Because of these variables and lack of consensus among neutron radiobiologists, AFRRI will convene a symposium on the Biologic Effects of High-Energy Neutrons in the spring of 1982. Subsequent AFRRI fast-neutron work will be predicated on the results of this symposium.

THE THREAT

Numerous studies address the nuclear threat faced by the United States and its allies. The nuclear threat is primarily from the U.S.S.R. and Warsaw Pact countries. The threat of strategic nuclear war has now been expanded to include the tactical use of theater nuclear weapons (1). The rationale for the use of tactical nuclear weapons is provided by former Chairman of the Joint Chiefs of Staff, General Brown (2).

> "A theater nuclear capability is indispensable to successful deterrance and defense. Theater nuclear forces complement general purpose forces and provide a continuum between conventional and strategic nuclear forces.
>
> "In the event aggression cannot be contained conventionally, theater nuclear forces provide the capability to terminate, if necessary, a conflict at less than a strategic nuclear level of intensity, on terms acceptable to the United States and its allies. Tactical nuclear weapons are deployed as an integral part of our theater forces to strengthen the deterrent effect of forward defense and to provide immediately available combat power to augment conventional forces. In the case of a Warsaw Pact attack which allows NATO little time for preparation or mobilization, NATO conventional forces would be greatly disadvantaged. A credible option for selective employment of theater nuclear weapons can contribute to deterrence and also provide augmentation should conventional means be found insufficient.
>
> "The conventional balance in Europe is such that if the Warsaw Pact forces are able to mass secretly and apply armored pressure to a given point in the defense line, NATO's ability to defend with conventional forces would be greatly weakened. Selective employment of nuclear weapons against armored thrusts could greatly contribute to theater deterrence and provide an intermediate option between conventional warfare and a general nuclear war.
>
> "The Soviets currently possess a tactical nuclear capability which could serve as a significant reinforcement to their offensive operations in Europe."

A comprehensive review of Soviet Theater Nuclear capability by Douglass (3) suggests that theater nuclear weapons are an integral part of the Soviets' combat capability. They plan an in-depth, massive, surprise nuclear strike coordinated with air and ground exploitation. In addition to the increasing possibility of theater nuclear war in both Europe and the Middle East, international terrorism may result in nuclear casualties. In light of these factors, this plan has been developed.

OVERVIEW OF MILITARILY RELEVANT RADIOBIOLOGY

Detonation of a nuclear weapon is an extremely complex event that releases a large amount of energy with multiple hazards for anyone within the weapon's range. For tactical nuclear weapons, there is a significant area in which radiation is the primary threat to combat personnel. Exposure to ionizing radiations can produce radiation injury that varies in severity, scope, and course, depending on conditions of exposure.

Signs and symptoms of radiation sickness after exposure to penetrating whole-body radiation are characterized by three successive phases. The first is a transient, prodromal phase, which can develop within minutes after exposure. The second is described as a latent period in which an individual may be relatively free of symptoms. The third and main phase of radiation illness ends in death for individuals who have received lethal doses of radiation.

The dose required to produce the prodromal phase in man appears to vary widely, depending on individual differences and susceptibility. Information on this subject is meager, deriving almost completely from data from accidental exposures, patient irradiations, and reconstructions of the Japanese experience at Hiroshima and Nagasaki. Several attempts have been made to describe the relationships between radiation dose, time, and symptoms. Minimal data are available on the occurrence of prodromal symptoms as a function of time after irradiation, for doses of 100-300 rads. The combat ineffectiveness produced by these symptoms within this dose range has never been fully assessed. Doses above 300 rads are generally considered to be lethal for more than 50 percent of exposed individuals. Review of the impact of sublethal radiation doses on combat, based on the human information currently available, is under way by Defense Nuclear Agency. Since these signs and symptoms have never been fully defined as to their impact on combat operations, none of the early effects of radiation injury (except early transient incapacitation) have been quantified in terms of their ability to produce combat ineffectiveness.

The second phase, or latent period, is a relatively symptom-free interval between prodromal manifestations and manifestations of the main phase of radiation illness. Except for lethal damage to the central nervous system (which occurs at doses above 10,000 rads and develops within minutes after irradiation), the latent period represents the time required for depletion of cells in affected tissues through interference with the cell-renewal process.

The third or main phase of the acute radiation syndrome is marked by symptoms such as fever, loss of appetite, lassitude, fatigue, weakness, loss of weight, diarrhea, infection, hemorrhage, skin reddening or tanning, loss of hair, shock, inability to walk, agitation, disorientation, convulsions, and coma. Severity of the main phase of radiation sickness depends largely on the number of stem cells surviving in mitotically active tissue, since stem cells are the only source of functional cells available for perservation or restoration of tissue integrity.

At or below midlethal dose levels of whole-body radiation (approximately 300 rads), the killing of hematopoietic stem cells is the principal cause of radiation illness. The physiological consequences of damage to bone marrow include the increased susceptibility to infection, bleeding, and anemia as well as the lowering of immunity. The hematopoietic form of radiation injury may be manifest between 3 and 8 weeks after exposure, with the greatest frequency occurring at 4-5 weeks after irradiation. Irradiation interferes with the renewal of cells lining the digestive tract. These cells are short-lived, and must therefore be renewed at a high rate. Radiation of sufficient magnitude to interfere with their renewal leads to their depletion within a few days. Because the rate of cell turnover is highest in the small intestine, cell depletion occurs sooner in this part of the gastrointestinal tract. The physiological consequences of gastrointestinal injury from radiation will vary, depending on the region and extent of damage. The radiation responses of the stomach, colon, and rectum are similar to that of the small intestine, but they occur more slowly. Responses of the mouth and esophagus closely resemble that of the skin. Exposures of the entire abdomen to 2000 rads or more of radiation can lead to death in less than 4 days, from loss of intestinal lining and resultant loss of electrolytes. Exposure to lower levels of whole-body irradiation may lead to ulcerative inflammation of the digestive tract, which occurs in association with the hemorrhage and infection resulting from damage to bone marrow.

The earliest symptom of radiation injury to the skin is reddening, which may be followed by blistering, ulceration, or sloughing. However, the longest and most intense of the radiation effects on the skin may not occur until about 10 days after exposure, at dose levels above about 1000 rads. Reddening of the skin may occur within hours. Severe effects of the skin, which require weeks to develop, occur only after massive exposures; they are not manifest at doses below 1000 rads. The brain is the most radioresistant tissue in the body. Death due to direct injury of the brain is now thought to occur only at doses in excess of 10,000 rads. At these doses, damage of the brain is marked by multiple small hemorrhages in the gray matter and white matter and an abnormally great amount of fluid surrounding the brain tissue. At these high doses, death, accompanied by tremor, loss of motor ability, and convulsions, can occur within hours after irradiation. This type of radiation death is generally defined as the central nervous system syndrome. When the dose is below the level required to produce the central nervous system syndrome and above the dose level required to produce the gastrointestinal syndrome, then death is thought to be due to cardiovascular failure.

For most of the effects discussed thus far, a given dose of radiation is more damaging when it is absorbed in a single, brief exposure than when it is absorbed in many exposures or in one exposure at a low dose rate. This difference in effectiveness is generally attributed to recovery from radiation damage during irradiation. If the dose rate is low enough, the effects caused at the onset of exposure may be repaired by the time the remainder of the radiation is being delivered. Two types of recuperation from radiation injury are postulated: (a) recovery within cells and (b) replacement of or compensation for injured and dying cells. The damage produced by radiations of high linear energy transfer (LET), such as alpha particles and fission neutrons, is less dependent on dose rate than is the damage produced by low-LET radiations, such as X rays and gamma photons. Estimations used to define lethal effects of irradiation under differing

exposure conditions are complicated by our ignorance of both the appropriate values for recovery half times and the fraction of radiation injury that is reparable. The irradiation of the whole body causes greater injury than irradiation of part of the body. In general, indirect effects on unexposed tissues are relatively unimportant when compared to the direct effects on exposed tissues. Shielding only a small part of these tissues can substantially protect against death. The exception to this generalization is early transient incapacitation, which can be produced by irradiating either the head or the trunk of an individual. However, as in other effects, the degree of incapacitation is apparently related to the amount of tissue irradiated. Attempts to determine the relationship between dose and lethality for man and other large animals is complicated because the radiation is not distributed uniformly throughout the body. Thus, it would be more difficult to predict the median lethal dose for man for a free-in-air dose or a mid-epigastric dose than for a bone marrow dose, since the midlethal whole-body dose depends on injury to the blood-forming organs within the bone marrow. Likewise, a mid-head dose may be more appropriate for predicting the early incapacitating effects of radiation than is a bone marrow dose. Relating dose to radiation effects in man and other large mammals is further complicated by the fact that mixed-spectrum radiations change as they interact with body tissue. This change in quality of a mixed-spectrum field is significant since the biological damages produced by high-LET and low-LET radiations are not equivalent. High-LET radiations such as alpha particles or fast neutrons are generally regarded to have a greater relative biological effectiveness than low-LET radiations such as X rays and gamma photons. The one exception to this generalization that seems to be significant in predicting the effects of ionizing radiation on combat personnel is that gamma photons have been found to be more effective in producing early transient incapacitation than either high-energy neutrons or fission spectrum neutrons.

The effectiveness of low-LET radiation in producing biological damage varies significantly with the oxygenation of exposed cells. Based on this principle, a number of materials were tried during the early and mid-1960's to produce radiation protection by lowering the oxygen tension within tissues. Other chemicals, which act as anti-oxidants or radical scavaging agents, have been evaluated as radioprotective substances. As in the case of hypoxia, the protective action of these chemicals depends on their presence at the time of irradiation. Although some of these drugs have been found to increase the median lethal dose for X rays by a factor of 2 to 3, all the materials tested today, with the possible exception of WR2721, have been too toxic to be used routinely in humans.

NUCLEAR WEAPONS EFFECTS EDUCATION

The objective of the Education Program of AFRRI is to improve the operational readiness of our forces through dissemination of the latest available information on the medical aspects of nuclear weapons. Currently, a 1-week course is given biannually at AFRRI on the Medical Aspects of Nuclear Weapons. Approximately 30 hours of lectures are presented by about 25 speakers. The faculty is primarily AFRRI personnel, augmented by outside medical experts on burn and radiation injuries. This program is attended by about 150 persons each year.

The 1-week course is modified to meet the needs of audiences outside AFRRI, who are primarily military medical professionals, notably physicians. Courses varying in length from 4 hours to 3 days have been presented by AFRRI personnel to a variety of active and reserve military groups in CONUS and in Europe. A special 14-hour block of instruction has been prepared and taught to first-year medical students at the Uniformed Services University of the Health Sciences. All courses are supported by a text prepared by the speakers.

Over the past year, there has been a marked increase in the number of requests for speakers from AFRRI to present mini-courses on the Medical Aspects of Nuclear Weapons, and this trend is expected to continue in the near future. It is expected that the presence of large numbers of nuclear weapons in the hands of both friendly forces and potential adversaries will require AFRRI to be involved in similar educational programs over the next 5 years.

DEGRADATION OF COMBAT PERFORMANCE

EFFECT OF IONIZING RADIATION ON COMBAT EFFECTIVENESS

The U.S. Army, U.S. Navy, U.S. Air Force, and Defense Nuclear Agency (DNA) have all published requirements for research information that would allow reliable prediction of the effect of ionizing radiation on human performance. The Army has a requirement to know the dose/task relationship that produces casualties for typical military tasks (4). Specifically, the requirement is to know the radiation levels that cause both temporary and permanent combat ineffectiveness in 50 percent of a population within 5 to 10 minutes after a nuclear detonation, in the tasks of checking out and launching a missile system; loading and firing a large-caliber weapon; running, aiming, and firing a rifle; and operating a vehicle.

The Navy defines a technical need for information on performance decrement in the areas of superlethal and low-level radiation exposure (5). The extent to which personnel are incapacitated or degraded in their performances is especially important in complex tasks such as data processing or decision making and in operator tasks that require sensory, perceptual, motor, or verbal responses. The Navy defines specific technical needs for two areas: (a) information on the degree to which performance degradation affects trained personnel in the critical functions listed above; and (b) research to evaluate the effects of radiation exposure on vision, hearing, touch, equilibrium, data sensing, and motor response.

The Air Force biomedical research requirements state that the effects of ionizing radiation on human performance are of particular interest to the survivability/vulnerability analysis of weapon systems (6). Predictions of performance of an air crew and subsequent estimates of mission success are needed by the Strategic Air Command, Tactical Air Command, and Aerospace Defense Command for force structure, target assignment, tactics, and route assignment. The Air Force defines an urgent need for better prediction of performance for manned weapons systems operating in nuclear environments, especially dynamic performance, which appears to be essential in the majority of Air Force operational systems.

The DNA Working Group on Nuclear Radiation Effects on Ground Combat Units defined several requirements for research relating to the effects of ionizing radiation on the degradation of performance (7): (a) documentation of the effect of prompt dose on efficiency in performing a judgmental, cognitive, or discriminative task; (b) extension or continuation of studies on the role of stress in the response to nuclear radiation; (c) investigation of the influence of fatigue on the response to radiation; (d) greater understanding of the degradation and incapacitation response as a function of dose level and time after radiation; (e) greater understanding of the delay of response after a prompt radiation dose.

In addition to these research requirements for information on the direct effects of radiation on task degradation, the Air Force has a requirement for research concerning nausea and vomiting after radiation doses below 1000 rads. This requirement is for the determination of expected incidence,

duration, frequency, and severity of radiation-induced emesis, because postirradiation vomiting is as severe a problem as incapacitation, for a pilot who is either relying on a chemical defense ensemble or is engaged in a critical maneuver.

As an extension of the research requirements for information on the effects of radiation on the individual, the military services have a need to know how radiation affects the performance of combat crews and units. The Army requirement is for information on the integrated response of crews (e.g., tank crews) when one or more individuals in a crew suffers from performance degradation or radiation sickness. The Air Force emphasizes the requirement of developing a multi-man performance model for analysis of strategic, tactical and command, and control and communication systems, in which man functions as an essential system element. The DNA Working Group on Nuclear Radiation Effects on Ground Combat Units recommended research to assess the influence of slight degradation in performance on military unit or team effectiveness.

To meet these requirements, a comprehensive set of data derived from human and animal exposures will have to be assimilated. This should permit prediction of the degree of degradation that can be expected in both the behavioral and physiological responses to irradiation during the first weeks after a nuclear detonation, as a function of radiation dose, quality, and dose rate. At militarily relevant levels, little is known directly about the effects of ionizing radiation on human performance. What we do know about this behavioral response comes from the analysis of radiation accidents and some inferences from the Japanese experience at Hiroshima and Nagasaki (8,9). Both of these sources of information are entirely qualitative in nature. There have been some efforts to administer standardized tests to persons receiving clinical irradiations (10,11). There are few of these studies, and they are limited in their value to military planning because the radiations were not prompt, whole-body, mixed-spectrum exposures but usually protracted, partial-body exposures in which the subjects were always seriously ill with neoplastic disease and often being treated simultaneously with other regimens, including chemotherapy. Thus, almost all the systematic, quantitative data on radiation-induced performance decrement have been derived from animal experiments.

The focus of animal studies has been incapacitation of the subhuman primates, since the incapacitation response has military relevance and the response of primates seems most like man's response after acute whole-body irradiation. Performance decrement in the monkey has been evaluated for numerous behavioral tasks after whole-body and partial-body irradiation, for various radiation qualities and dose rates (12-48). Several generalizations have emerged from those studies. (a) Early transient incapacitation is qualitatively very similar for many behavioral tasks. (b) The frequency of incapacitation within a population increases as a function of radiation dose. (c) Incapacitation can be elicited by either trunk-only or head-only irradiation. (d) Neutrons are less effective in producing early transient incapacitation than are gamma photons. (e) The frequency of incapacitation produced by a given radiation dose is proportional to the demands or stress of the task being performed. These findings and the data they represent are the basis for the current combat casualty criteria (49). The present criteria are based on the incapacitating dose levels for both physically demanding tasks and

undemanding tasks. They do not include combat ineffectiveness due to partially degraded performance that may result from slower reaction to the task, task stress, or prodromal effects of the acute radiation sickness. Information on the degree of performance degradation resulting from these factors is not presently available in sufficient detail to permit its use with confidence. Radiation-produced degradation for specific combat tasks and the threshold for radiation effects on perceptual and complex behavioral functions are presently unknown.

Several disciplines and approaches must be integrated in order to fulfill the military requirements for information on performance degradation of both individuals and units, due to the effects of ionizing radiation on behavior and physiology as a function of radiation dose, quality, and dose rate as well as time after exposure. Since human data on radiation effects are few and since animal experiments cannot be comprehensive models of human performance, confident predictions of radiation-induced combat ineffectiveness can be derived only from the application of data on the degradation of specific functions to actual task analyses of critical military tasks. This approach to predicting combat ineffectiveness has several advantages over the design of empirical animal experiments to address each combat task. (a) Specific task functions (e.g., visual discrimination, tracking, or running) can be defined that are amenable to testing with animal models. (b) Animal models are best suited to defining degradation of specific functions. (c) Although few human data exist for radiation-induced task degradation, physiological data, which could be combined with animal data, do exist for certain radiation effects. (d) The performance decrement for each critical combat task can be made specific to that task. (e) Unit (e.g., tank crew) effectiveness may be assessed by applying the degradation data across all the tasks within the unit. (f) Often the detailed task analyses for critical military tasks already exist. (g) This approach provides the basis for developing overall models for combat ineffectiveness.

The approach in meeting the requirements for information on radiation-induced performance decrement will be to: (a) carry out specific animal experiments to quantify radiation-produced degradation for those critical combat functions for which data do not exist (e.g., threshold for radiation-produced decrement), (b) quantify the frequency and duration of prodromal radiation effects (e.g., nausea and vomiting) for midlethal to sublethal doses, (c) establish descriptive models for radiation degradation functions, (d) apply degradation data developed from the experiments to predict combat ineffectiveness, and (e) combine degradation analyses for crews in order to predict unit ineffectiveness.

Work in this area over the next 5 years will focus on four areas: (a) development of more sensitive behavioral assays for low-dose radiation effects, (b) completion of experimental work with primates to evaluate interaction of combat-induced and radiation-induced fatigue, (c) initiation of an experimental effort to define the threshold for the effect of radiation on behavior, and (d) modeling and analysis. The results of the analysis and modeling effort will be a major input into a Comprehensive Personnel Nuclear Weapons Effects Model (CPNWEM). The CPNWEN will be developed over a number of years. It will be designed as a data base and repository, with algorithms to answer questions on both the operational and medical effects of nuclear weapons. The CPNWEM will begin by building on

the existing AFRRI Primate Behavioral Data Base and the descriptive algorithms that have been developed for those data.

Milestones for Ionizing Radiation Effects

FY83 - Complete the work to establish the median effective dose for the interaction of cognitive and radiation fatigue. Establish the overall approach for the CPNWEM. Establish the preliminary data base for early transient incapacitation, permanent complete incapacitation, and acute radiation sickness. Establish the time dependency of these effects as a function of radiation dose and quality for nonhuman primates.

FY84 - Complete the experimental work to establish the median effective dose for the interaction of physical and radiation-induced fatigue. Define operational military tasks to be included in the CPNWEM (e.g., loading and firing a large-caliber weapon or aiming and firing a rifle), and obtain task analyses of these tasks.

FY85 - Begin the experimental work to determine the threshold for radiation effects on performance of military tasks. Add the data derived from the study of the interaction of fatigue and radiation to the CPNWEM. Apply the information from the CPNWEM to the evaluation of degradation of operational military tasks for which task analyses have been developed.

FY86 - Continue the experimental studies to determine the threshold for the effects of ionizing radiation on performance. Develop algorithms for the prediction of radiation-induced combat ineffectiveness, and validate those algorithms.

FY87 - Complete the experimental work to determine the threshold for the behavioral effects of ionizing radiation on behavior. Include the threshold data into the CPNWEM, and apply the data to the algorithms developed to predict low-dose effects of ionizing radiation on combat effectiveness.

MECHANISMS OF PERFORMANCE DECREMENT AND INCAPACITATION

The highest priority requirement for radiobiology research in the present Army QRR is for verification of the reliability of the extrapolation of primate response to radiation in determining the incapacitating doses for humans (4). Presently, the Army assumes that the response of man to nuclear radiation is equivalent to that of a primate when equal doses are received (rem) to the mid-head position. Research is necessary to verify the reliability of this extrapolation from primate to man and to confirm the dose/task relationship in casualty production. Failure to extrapolate correctly from primate to man will cause incorrect casualty estimations.

The Air Force has a research requirement for neurophysiological responses and performance decrement after exposure to ionizing radiation (6). Important factors include hypotension, cardiovascular response, and cerebral ischemia. Information on the correlation of physiological changes (especially neurophysiological responses of specific sensory and motor systems) with performance decrement and also on the occurrence and duration of transient and permanent incapacitation is needed, to form the basis of

protective and therapeutic "man-hardening" techniques to enhance post-exposure crew performance.

Understanding the physiological basis for radiation-induced incapacitation is essential in order to provide protection against these radiation effects, to verify the extrapolation of experimental animal results to man, and to develop general models of radiation-produced performance decrement. In radiation injury, as in other toxic insults to the body, there are two generally accepted approaches to the extrapolation of data from lower order animals to man (50,51). One approach requires knowledge of the biological mechanism that produces the injury, and the other approach requires establishment of an orderly progression of effect from lower order animal species to man as the basis for extrapolation. The occurrence of early transient incapacitation (ETI) in man and the dose at which it occurs are impossible to establish unequivocally since there is only one case in which a person received a prompt, whole-body, mixed-spectrum dose and ETI occurred (52). Radiation-induced ETI clearly occurs in the subhuman primates. This phenomenon has been studied fairly extensively, and these data are the basis for the current combat casualty criteria. The occurrence of ETI has been demonstrated in several primate species, including the Stumptail, Rhesus, Cynomolgus, Spider, and Squirrel Monkeys and the Baboon (53). In all cases, incapacitations for these animals are qualitatively very similar. Miniature pigs are incapacitated by radiation intensities comparable to those needed for Rhesus Monkey incapacitation, but there are marked qualitative differences in these two species. Pigs are immediately incapacitated and have opisthotonic convulsions, whereas monkeys are incapacitated several minutes after irradiation and exhibit no convulsions until extremely high dose levels are reached (54). Rats also exhibit ETI, but the dose required to produce the response is nearly one order of magnitude higher than that required for the primate (55). The response of the dog to supralethal doses of radiation is neither qualitatively nor quantitatively similar to the primate, miniature pig, or rat. Thus, there does not appear to be an orderly progression in the radiation-produced ETI among the animals that have been most fully studied. The occurrence of incapacitation in man seems very likely, since incapacitation does occur in the rat, miniature pig, and subhuman primate. This qualitative similarity in response, particularly among the primates, suggests that the one case of incapacitation that has been observed in man is likely to occur in other similarly irradiated persons. However, it is not possible to quantitatively predict with certainty the dose at which ETI would occur in man, because of the lack of an orderly dose progression within the lower animal species. In the face of this, one must rely on determining basic mechanisms as the basis for validation of primate data to use in predicting man's response to radiation.

Research to establish a physiological mechanism for ETI has concentrated on studies of central nervous system (CNS) electrophysiology, blood pressure, and global cerebral blood flow (56). Gross electrophysiological studies of the CNS have provided no insights into the mechanism for ETI because this approach is not specific enough to differentiate between the sites of damage (33). Since blood pressure can fall dramatically after irradiation, this hypotension has often been thought to be the cause for ETI. Using this explanation, brain dysfunction could be explained simply by an inadequate supply of oxygen to the CNS. Although the occurrence of hypotension after irradiation is highly correlated with the occurrence of performance decre-

ment, the experimental use of norepinephrine to maintain the blood pressure postexposure failed to prevent ETI (57). The disruption of the CNS function following hypotension is assumed to result from decreased cerebral blood flow. Since the CNS can often maintain cerebral blood flow under conditions of severe hypotension through autoregulation, the gross cerebral blood flow has been measured directly in irradiated animals. These studies have produced mixed results, and thus have not been conclusive in establishing the role of cerebral blood flow in producing incapacitation (58,59). The release of histamine in the body after irradiation has been the biochemical mechanism most studied as the potential cause for ETI. The prevention of histamine release after irradiation with antihistamines has proved beneficial in preventing postirradiation hypotension. However, antihistamines do not prevent ETI (60).

A neurochemical approach has provided valuable clues as to possible neurotransmitter candidates underlying ETI. Studies of dopaminergic and cholinergic activity in the caudate nucleus suggest that the release of both transmitters are elevated and follow the time-course of ETI (61). In addition, the cyclic nucleotides, adenosine-3',5',-monophosphate and guanosine-3',5'-monophosphate, are both reduced during ETI in several areas of the brain (302). This effect is not related to an action of radiation on the enzymes of synthesis, or degradation of these compounds (63). These changes in neurotransmitter function may be related to motor decrement observed during ETI.

To determine the mechanism for ETI, the following factors will be investigated: hypotension after irradiation, changes in membrane permeability to calcium flux, changes in CNS neurotransmitter release, changes in monosynaptic reflexes, changes in mechanisms of metabolic ionic recovery of both neuronal and glial cells, measurements of alteration in neuronal and glial interactions, and studies of the mechanisms of damage produced by chemicals that simulate radiation-produced incapacitation. Invasive techniques with implanted electrodes will be used to measure regional cerebral blood flow (RCBF). Non-invasive techniques--using radionuclides, multiple arrayed detectors, and single photon emission computed tomography (SPECT)--will also measure RCBF. In addition, localization and quantitation of muscarinic receptor labels will be studied using SPECT, and correlated with the quantitated RCBF.

Milestones for Performance Decrement and Incapacitation

FY83-85 - Determine the role of cardiovascular shock, muscarinic receptors, neuronal activity, and motor synapses in producing early transient incapacitation.

FY86 - Establish the relationship of changes in synaptic transmission, CNS neurotransmitter release, and intracellular metabolism related to performance decrement.

FY87 - Evaluate the direct chemical protection of the nervous system and the systemic radioprotective substances for prevention of emesis and ETI.

EFFECTS OF DRUGS ON COMBAT PERFORMANCE

Drug-drug interactions are modifications of the effect of a drug when used in conjunction with another. These interactions may be additive, subtractive, or synergistic, and they account for a significant proportion of all drug reactions. The Boston Collaborative Drug Surveillance Program found a 5 percent incidence of adverse drug reactions in in-patient medical services (64). Many of these advese drug reactions were the result of the simultaneous administration of routinely used drugs. The time course of drug activity is determined by its pharmacokinetic properties, and it includes the absorption, distribution, metabolism, and excretion of the drug. Multiple administration of drugs may saturate a person's ability to metabolize or excrete them, which may significantly alter that person's visual ability. In addition to the above, the effects of sex, age, physiologic factors, and psychologic factors are also important.

The integrated battlefield poses a significant hazard not only because of the potential effects of chemical, nuclear, and biological agents coupled with traumatic injuries but also because of the self administration of a host of drugs: radioprotectants, antiemetics, chemical antidotes, vaccines, over-the-counter drugs, alcohol, and recreational drugs. During the last few decades, extremely toxic organophosphates have been developed. These irreversible anticholinesterases (tabun, sarin, soman) in addition to a host of other agents have entered the arsenals of NATO and Warsaw pact countries. To provide an antidote and reverse the neuromuscular paralysis, the cholinesterase reactivator Pralidoxine-Chloride (2 PAMCL) has been developed. This agent and atropine sulfate have been suggested by the U.S. Army as possible self-administered antidotes (65).

All known antidotes or therapeutic drugs may produce side effects. The cyclopolegic effect (near vision) has made atropine unacceptable for use by aircrews. Atropine also affects thermal regulation in addition to other central disturbances (66). The U.S. Army Institute of Environmental Medicine will shortly study the effects of these drugs (with thermal stress) on performance in human volunteers with informed consent (67). There are no significant data or studies of WR2721 (radioprotectant) or antiemetics on performance. A recent cancer therapy trial with WR2721 revealed 2 of 50 patients had a profound hypotensive response to WR2721 (68). In addition, 29 of 50 experienced nausea and 22 of 50 experienced vomiting. These were usually encountered at doses greater than 170 mg/M^2.

Emesis is a serious complication of radiation exposure for aircrew members with masks or for anyone wearing a chemical ensemble. Recognition of this has prompted significant efforts to evaluate the efficacy of various agents in preventing radiation-induced emesis (69-71). Many of the most effective drugs have significant side effects.

It is proposed that the most efficacious agents from each group be studied for physiologic and behavioral dysfunction.

COMBINED EFFECTS ON COMBAT PERFORMANCE

Army QRR 14.4Q1 describes the military relevance of combined injury:

> "The impact of exposure of personnel to a nuclear detonation combined with other external trauma (injuries, infection, chemical and biological agents, and psychological stresses) on the ability of a man to perform his prescribed tasks is unknown. Knowledge of these factors will allow valid predictions of the combat effectiveness of troops exposed to various environments on a nuclear battlefield. Currently, personnel risk levels do not take into consideration these integrated effects, and combinations of these stresses may greatly influence establishment of personnel risk (troop safety) criteria for friendly combat personnel." (4).

The interaction of nuclear weapons effects and other battlefield trauma must be determined in order to develop diagnostic and therapeutic means to counteract the increased casualty load expected. Lack of this information could handicap medical support of casualties on a nuclear battlefield.

In addition to blast, thermal, and ionizing radiation, other factors such as chemical and biologic warfare agents and nonionizing radiation are very likely to be present in a combat environment. It is therefore of great importance to determine the impact of these agents in combination with ionizing radiation on the overall response of personnel and the effectiveness of these agents in enhancing early and late effects of ionizing radiation. Since, in a future combat environment, combined or multiple injuries will be more predominant than radiation damage alone, it is important to study the physiologic and biochemical mechanisms involved in order to develop proper medical counteractive measures.

Chemical agents, such as the various anticholinesterases, produce their biologic effects by inhibiting the enzyme acetylcholinesterase, and thus prevent the destruction (hydrolysis) of acetylcholine. This results in its accumulation in the synaptic space, which is the site of action of this neurotransmitter.

Anticholinesterase agents act on the autonomic system, mainly within the central nervous system. It is known that their antidotes (P2S, atropine, pyridostigmine, etc.) do not, or barely, cross the blood-brain barrier in order to counteract or protect the organism from the deleterious effects of these agents. Therefore, development of techniques to increase penetrability of the blood-brain barrier into the brain of these antidotes (as well as radioprotectants such as WR2721 which do not penetrate the blood-brain barrier) is of paramount importance for the protection and treatment of military personnel exposed to ionizing radiation alone or in combination with chemical agents.

Preliminary experiments in the Biochemistry Department at AFRRI have shown that a brief exposure of rats to low-level microwave radiation, of power density not exceeding the accepted safety threshold, results in reversible "opening" of the blood-brain barrier (72). Therefore, it should be important to determine if this observation can serve as the basis for development of techniques to increase the penetrability of radioprotectants and other antidotes into the brain, for more effective protection or treatment of irradiated personnel.

In comparison with other tissues, the central nervous system (CNS) is resistant to ionizing radiation. Yet subtle functional changes do occur with relatively low levels of exposure (73-75). Within the first week after irradiation, there appears to be an increase in CNS excitability. Subsequently, neural activity may actually fall below normal levels (76,77).

Enhanced activity of the brain is reflected in changes that can resemble epileptiform discharges. Following 200 rads whole-body X irradiation in cats and rabbits, hippocampal spiking was observed (75,78). Timiras et al. (75) found that 250 rads whole-body X irradiation mimicked the actions of the convulsant pentylenetetrazol (PTZ), by increasing the electrical activity of the prepyriform cortex in rats.

Exposure to ionizing radiation tends to increase susceptibility to seizures. The frequency of audiogenic seizures in mice is enhanced following chronic exposure to low levels of radiation (73,79). The threshold for electroconvulsive seizures is reduced in adult rats (77) after as little as 25 rads X irradiation (80).

Since the general level of excitability of the nervous system is altered by irradiation, pharmacologic and toxic agents that affect the CNS could be altered in their effectiveness. Up to 3 days after irradiation, mice were more resistant to the effects of the anesthetics chloralose and pentobarbital (81) and the analgesic morphine (76). Subsequently the analgesic activity of morphine was increased (76). Conversely, convulsant agents are more effective immediately after exposure to radiation (82). Barnes observed that 10 rads X radiation increased the lethality of pentylenetetrazol in adult mice tested within an hour. However, 13 days after exposure to 500 rads X radiation, adult rats were less susceptible to PTZ-induced seizure activity (82). Similarly, 1 week after exposure to ionizing radiation, the threshold for picrotoxin-induced seizures was increased (83). The time course of changes in drug sensitivity follows the time course of changes in nervous system excitability.

Many of the agents used in chemical warfare have CNS actions. These compounds include soman, sarin (GB), parathion (active metabolite, Paraxon), malathion, and DFP (disopropylphosphoro fluoridate). Soman and sarin are the most potent of the agents available. Peripherally these compounds act as anticholinesterases. The central mechanisms of action are currently under investigation at Edgewood Arsenal. Among the gross effects attributable to CNS mechanisms is induction of convulsant activity. Since radiation acutely enhances the excitability of the CNS and alters the brain sensitivity to some centrally acting compounds, the central effects of agents utilized for chemical warfare would probably be altered.

The isolated nervous system of Aplysia californica provides an excellent preparation for the investigation of direct effects and interactions of ionizing radiation and chemical warfare agents in neural tissue. The nervous system of Aplysia consists of several ganglia, some of which are paired. The neurons are large and easily impaled with several microelectrodes. Identified neurons have characterized ionic responses to a variety of neurotransmitters. These properties allow a well-controlled analysis of changes that occur after irradiation and exposure to chemical agents. This work will be

done in collaboration with Margaret Filbert at Edgewood Arsenal and the Radiation Sciences Department at AFRRI.

In experiments performed to date, one half of each of the paired ganglia was exposed to up to 185,000 rads gamma in the AFRRI cobalt-60 facility. These ganglia, along with their naive counterparts, were driven to the Biomedical Laboratory, where facilities to handle chemical warfare agents are present. The naive and exposed ganglia were pinned out in a lucite chamber, and the same identified cell from each was penetrated with microelectrodes. Responses from the cells were elicited by ionophoretic application of putative neurotransmitters or electrical stimulation. Membrane potentials were lower and less stable in the radiation-exposed cells. Addition of soman to the perfusate caused a depolarization, without firing, only in those cells that had been irradiated.

Milestones for Neurophysiologic Techniques

FY83 - Determine dose-response relationship of cobalt-60 on neurons from isolated ganglia.

FY84 - Determine dose-response relationships of various chemical agents, including DFP, soman, and sarin, on neurons from isolated ganglia.

FY85-87 - Determine changes in ED50's of both chemical agents and radiation when applied in concert with different quality radiations.

Many drugs cause highly significant physiological derangements when these agents bind to receptors. Many of the most potent receptor agonists and antagonists (vide supra) are the chemical warfare agents. Quantitative analysis of receptor binding in vivo pre- and postirradiation and/or chemical agents may provide understanding of their mechanism and interaction.

Muscarinic cholinergic receptor binding has been identified biochemically in vitro by cholinergic agonist binding (84). Quinuclidinyl benzilate (QNB) is a potent peripheral and central muscarinic antagonist (85,86). The distribution of muscarinic acetylcholine receptor antagonists has recently been demonstrated at AFRRI (87). These studies have involved in vivo labeling but in vitro quantitation of the receptor labeling. The Soviet Union has been very active in this area of research and has shown a significant increase in cholinergic receptor (another acetylcholine-mediated receptor) activity in rat brain and heart after 350 rads total-body irrdiation (88).

The ultimate goal is to quantitate the changes in muscarinic receptors totally in vivo so that the time course of these receptor changes and correlation with behavior can be made. Significant progress has recently been made in this area at AFRRI (89). The receptor antagonist (QNB) has recently been radiolabeled with iodine-123, a nearly ideal photon emitter. We have demonstrated the capability of imaging the muscarinic receptor distribution in the brain, heart, lung, liver, and stomach (90). The recent acquisition of a single photon emission computerized tomography (SPECT) system will allow quantitative evaluation (in vivo and noninvasively) of muscarinic receptors before and after irradiation, anti-cholinesterase active agents, and combinations of both.

Technical Approach

Use radiolabeled muscarinic antagonists and SPECT technology to quantitate the changes in activity in the autonomic receptors when animals are exposed to ionizing radiation and chemical warefare type agents.

Milestones for Muscarinic Receptor Studies

FY83 - Validate the quantitative assessment of muscarinic receptors by radiolabeled muscarinic agents with SPECT.

FY84 - Evaluate the changes in CNS and the myocardial muscarinic receptors postirradiation with gamma radiation.

FY85 - Evaluate the changes in the CNS and the myocardial muscarinic receptors after exposure to chemical warfare type agents and neutron irradiation.

FY86 - Extend these observations to combined injuries (radiation plus chemical agent) in addition to beginning behavioral studies to correlate with these agents.

FY87 - Continue the previous studies but assess the impact of antidotes and radioprotectants.

Hyperthermia

Over the past 10 years, tremendous interest has been rekindled in the use of hyperthermia and radiation for tumor therapy. A significant body of data suggests the use of heat and radiation to kill tumor cells (91). These data are important to the military because of the possible radiosensitizing effects of hyperthermia secondary to physical exertion and/or wear of the chemical ensemble. In vitro and in vivo experiments show increasing cell kill with increasing temperatures. The time of exposure to heat and elevated temperature are closely related (92). The cytotoxic effects of heat and radiation have recently been demonstrated in several cancer treatment studies (93).

The U.S. Army Institute of Environmental Research has been actively investigating the adverse effects of elevated core body temperatures and subsequent performance degradation. The problem is so significiant that several cooling garments have been designed and tested. Any physical or chemical agent that increases radiosensitivty needs to be carefully reviewed in order to define its impact on casualty criteria, performance decrement, and survival. AFRRI will develop joint programs with Natick Laboratories to assess this problem and devise methodologies to attenuate these effects (94). Milestones will be developed in a Memorandum of Understanding when Command approval has been received.

PREVENTION AND TREATMENT OF RADIATION EFFECTS

RADIOPROTECTANTS: CHEMICAL AND PHYSICAL

The Army QRR 14.b1 delineates a specific military requirement for radiation protection:

> "Determination of protective measures, physical or chemical, which are organ/system specific and, therefore, provide higher levels of protections than those measures currently available." (4)

The first animal experiment that stimulated widespread interest in developing radioprotective drugs for humans was performed 30 years ago by Patt (95). Large doses of cystiene were given intravenously 15 minutes before a lethal dose of X rays. All pretreated rats survived and all nontreated rats died. It was immediately recognized that development of a radioprotectant drug for man could have significant military implications. A tremendous amount of research was conducted on the development of potential drugs, and the Walter Reed Army Institute of Research was instrumental in developing over 4400 compounds (96).

Initial radioprotective drugs had several limitations. They were protective only at paratoxic doses, their protection was of limited duration and limited potency, and they were ineffective orally. Ideally, the drug should be administered orally to provide long protection. It should have no side effects, it should be stable, and it should not be abusable. The best radioprotective compound developed thus far is the phosphorothioate WR2721. This compound protected mice, dogs, and rhesus monkeys from X or gamma irradiation, and it demonstrated modest protection against neutron irradiation in mice.

Yuhas demonstrated a dose reduction factor (DRF) of 2.7 against 30-day mortality in C57BL/6J mice (97). This is the highest DRF reliably reported for the endpoint of lethality. Additionally, WR2721 was better tolerated than other compounds, while providing the best therapeutic index. Primary disadvantages to WR2721 for self-administration by military personnel are its lack of adequate activity after oral administration and the fact that it does not cross the blood-brain barrier (97). Large doses of WR2721 are needed for protection of large animals after whole-body irradiation, and the drug may not be very effective against neutron irradiation. Breakdown products of this unstable drug may cause undesirable side effects. In order to further understand how radioprotectant chemicals and endogenous radioprotective molecules increase the tolerance of tissues or organisms to ionizing radiation, it is necessary to determine how the curently available agents affect the physiological and biochemical systems. It should also be possible to use a combination of agents to provide a better therapeutic index or provide better stability to WR2721 in order to reduce its toxicity. The mechanism of action of WR2721 is generally believed to be direct free radical savenging by this sulfhydryl compound, but other factors, such as physiological effects on blood circulation and oxygen tension, must be considered. The effect of WR2721, other sulfhydryl agents, and antioxidants on the immune system is also important. Current studies have shown that

WR2721 protects cell-mediated immunity in whole-body irradiated mice and may even stimulate the immune response in nonirradiated animals (98). Other immunomodulating drugs are also being studied as radioprotectants. Azimexon, a cyanoaziridine compound, stimulates hematopoietic progenitor cells, which may play a role in radioprotection (99). Azimexon has been proposed as a radioprotectant that can be given after radiation exposure (100). Special emphasis is therefore being given in current experiments to the use of a combination of radioprotectants and immunostimulants to reduce the known toxicity and side effects of WR2721.

Previous studies have shown that WR2721 induces changes in the lysosomes, cyclic nucleotides, and prostaglandins—systems that are closely involved with tissue injury resulting from exposure to ionizing radiation (101). Other studies (102) have further indicated that radioprotectants are effective not because they are free radiacl and oxygen scavengers, but because they alter enzyme levels and other materials in the cell. A simplified model of radioprotection by various agents involving induction of interferon has been proposed (103), and the role of interferon on hematopoietic stem cell stimulation and radioprotection is under study (104).

Radioprotective properties of various compounds on irradiated mammalian cells in culture are being studied, since radiation effects on biochemical systems can be studied relatively quickly in vitro. Damage to the cell can be inflicted directly on DNA in the form of strand breaks, crosslinks or base lesions, or on other cellular entitied such as membranes or enzymes. The repair enzymes (nucleases, polymerases, and ligases, play the greatest role in determining radiosensitivity or resistance of the cell to radiation and chemicals. It has been shown that WR2721 enhances DNA repair capacity of several mammalian cell lines after high doses of gamma and electron radiation (105). Work in progress is aimed at elucidating the mechanism of this enhanced repair by WR2721, and the effects of combined radioprotectant treatment is being assessed. If the events or mechanisms that are involved with radiation protection of cells and tissues can be determined, then more efective radioprotectants can be produced.

It is now recognized that histamine (106) is released postirradiation and plays a significant role in the systemic manifestation of radiation injuries (107). Many pathophysiologic responses appear to be mediated by histamine: early transient incapacitation (ETI), performance decrement (PD), hypotension, gastrointestinal dysfunction (GID), etc. (56,108). It has been shown that histamine antagonists, while not preventing them, can attenuate some of the effects of radiation (109).

A drug of great interest is sodium cromoglycate (Cromolyn). Asthma is a disease in which the mast cell releases mediators that precipitate bronchospasm (110,111). One of the primary mediators released is histamine. Cromolyn has been very effective in preventing mast cell release of these mediators by stabilizing the mast cell membranes. There is minimal toxicity associated with its use. Systemic mastocytosis is a disease involving neoplastic transformation of the mast cells (112). The systemic manifestations result from release of histamine by degranulated mast cells. The diarrhea associated with this disease has been controlled with Cromolyn. H2 receptor antagonists (cimetidine and metiamide) have been shown to prevent the acid hypersecretion associated with mast cell disease,

and they are significantly useful in preventing the gastrointestinal effects of radiation (113,114).

Dietary constituents may play a significant protective role against the effects of radiation. Rats were exposed to cobalt-60-gamma irradiation. Only 1 of 16 rats fed the usual food pellets survived this dose of radiation, and the mean survival time was 9 days. In contrast, 11 of 15 rats survived that had been fed an experimental elemental diet for 1 week before irradiation, and the mean survival time was 59 days. It was of no value to begin the experimental diet after irradiation. It appeared that the experimental elemental diet was associated with both an enhanced cellular proliferation in the blood-forming tissues and a better response to antigen stimulation (115).

Vitamin E has also been demonstrated to provide some radioprotective effect in experimental animals (116). Radiation damage to tissues is partially mediated through formation of free radicals. Since Vitamin E is a free radical scavenger, it was tested for its ability to protect against the lethal effect of total-body irradiation in mice. Recent work at AFRRI shows that mice fed several times the physiologic amounts of Vitamin E had improved survival postirradiation. Mice were irradiated with 850 centigray of cobalt-60. The 30-day survival was 10% in the control mice and 60% in the Vitamin-E mice (117). Stimulation of cell-mediated immunity and enzyme protection by Vitamin E, as mechanisms of radioprotection, are under study.

Technical Approach

AFRRI will also perform lethality studies with WR2721 and other related sulfhydryl compounds recommended by Walter Reed Army Institute of Research in cell cultures and in vivo. The agonist or antagonistic effects of diet and vitamin E will be assessed.

Milestones for Radioprotectants

FY83-85 - Evaluation of WR2721, dietary factors, and immunomodulators before gamma and mixed-field irradiation.

FY86 - Evaluation of the above after fast-neutron irradiation.

FY87 - Assessment of multiple combinations of radioprotectants before different qualities of radiation to maximize postirradiation survival.

RADIATION-INDUCED CARDIOVASCULAR DYSFUNCTION

The U.S. Armed Services require radiation data to predict rates of troop survival, required numbers of personnel, and level of performance degradation. Many mission-essential tasks require stamina and involve demanding physical and cognitive skills. Minimal decreases in cardiovascular function may result in substandard performance or potentially lethal performance. Data from two radiation accidents involving humans and information from several human and animal studies have shown significant perturbations in

cardiovascular function, which have resulted in coexistent ETI, performance decrement, or death. Damage to the cardiovascular system causes an increase in vascular permeability and an inability to maintain blood pressure (118), which leads ultimately to a radiation shock syndrome. Many metabolic factors, including perturbations in glucose metabolism or humoral agents such as histamine, are felt to be intimately involved in the derangements in cardiovascular function.

Technical Approach

Several animal cardiovascular models that respond like the human system will be evaluated by a variety of contemporary techniques to quantitate the dose-response relationship for a variety of radiation levels that result in ETI, performance decrement, or cardiovascular dysfunction. Short-lived radioisotopes will be injected and coupled to computerized imaging devices to noninvasively determine decrements in postirradiation cardiovascular function. Standard invasive techniques will also be used. Once the effects are quantitated, radioprotectants (WR2721) and histamine antagonists (such as Cromolyn and cimetidine) will be given to quantitate the response. In addition, the cardiovascular dysfunction will be quantitated by assessing the ability of the circulatory system to maintain homeostasis in the fact of the combined insult of radiation and hemorrhage. Perturbations in glucose metabolism will be determined in irradiated animals, and the effects of irradiation on several related hormones (vasopressin, insulin, glucagon, and endorphines) will be determined. Once these effects are quantitated, prophylactic intervention will be attempted. Endothelial integrity has recently been evaluated and quantitated noninvasively. Changes postirradiation will evaluated and effects of penicillamine, epsilon amino caproic acid (EACA), WR2721, and thyroid-releasing hormone (TRH) will be studied to determine if capillary permeability can be normalized postirradiation.

Milestones for Cardiovascular Dysfunction

FY83 - Quantitation of cardiovascular dysfunction, regional pulmonary perfusion, and regional cerebral blood flow after photon irradiation.

FY84 - Quantitation of cardiovascular dysfunction, regional pulmonary perfusion, and regional cerebral blood flow after neutron irradiation (fission). Preliminary studies with prophylactic agents, radioprotectants, and histamine antagonists. Determination of organ-specific RBE.

FY85 - Quantitation of cardiovascular dysfunction, regional pulmonary perfusion, and regional cerebral blood flow after mixed-field irradiation.

FY86 - Quantitation of the dose-reduction factors (DRF) of phophylactic agents after mixed-field and neutron irradiation.

FY87 - Quantitation of the dose-reduction factors of combinations of prophylactic agents for different qualities of radiation. Synthesis of findings and recommendations to operational units.

RADIATION-INDUCED GASTROINTESTINAL DYSFUNCTION

Once a soldier has been exposed to radiation, what can be done in the way of treatment? Radiation does its greatest damage to the rapidly proliferating tissues, such as the bone marrow and the epithelium of the gastrointestinal tract. The radiation damage to these tissues then leads to nausea, vomiting, diarrhea, anemia, bleeding, and infection. Loss in electrolytes takes place, and ulcerations in the digestive tract occur, which permit the entry of infectious bacteria into the peritoneal space. In addition, there is a dramatic reduction in lymphocytes and platelets, which lowers the ability of the organism to resist other infections and stop hemorrhage.

The more acute symptomatic effects occur in the gastrointestinal system, and they may quickly incapacitate the exposed soldier. Their onset and severity are to a considerable degree dose-related, and may be expected to occur within the first few hours postexposure. These effects would obviously interfere promptly with soldier and unit performance. Thus, it is of considerable importance to mission accomplishment if these attacks could be prevented or, if not prevented, at least attenuated or delayed in onset. Regrettably, there is little understanding of the mechanisms of postirradiation gastrointestinal dysfunction.

The role of the prodromal effects of ionizing radiation in producing performance decrement in military personnel is not clearly established, and the mechanism by which nausea and vomiting are produced by irradiation is also not clear. There are indications that vomiting may be triggered by irradiation of either the head or the torso of an individual (119), that the mechanisms by which these two types of vomiting are produced are different, and that neutrons and gamma photons differ in their ability to cause vomiting after irradiation of the head or the trunk (120). Further, the radiation antiemetic drugs that have been tested are not equally effective for neutron and gamma irradiation, and the drugs that protect against emesis do not protect against ETI (121).

Vomiting is a complex, multilevel physiological act that requires relaxation of esophageal and stomach smooth muscles and the concomitant contraction of skeletal muscles of the abdomen, thorax, and diaphragm. This response can be modified by drugs whose site of action is the chemoreceptor trigger zone (CTZ) in the lateral segment of the area postrema and the vomiting center (VC) found near the *fasciculus solitarius* (122). Vomiting can be initiated by chemical stimuli to the CTZ while the VC receives neural stimuli (autonomic) from the GI tract, vestibular apparatus, and possibly the CTZ. The precise etiology of this response has not been elucidated, but complex interactions participate in radioemesis (123). Studies performed at the USAF School of Aerospace Medicine showed the efficacy of chlorpromazine, promethazine, cemetidine, and thiethylperazine (124). They found the emesis dose for 50% (ED50) in the dog to be 170 ± 38.5 rad. This dose could be doubled with several of the agents after cobalt-60 irradiation. These studies have recently been extended to neutron irradiation using the AFRRI reactor, and results should be available soon. The primary disadvantage of many of the antiemetics is their neurologic side effects. Domperidone is a peripheral dopamine antagonist that has demonstrated efficacy in preventing vomiting secondary to a variety of situations: postoperative, chemotherapeutic, pediatric, migraine, and hemodialytic (125). Recent studies at AFRRI have demonstrated the prevention of radioemesis in 90% of dogs

exposed to 800 rads TBI of cobalt-60 (71). These observations will be extended to the primate, and will include exposures to fission spectrum neutrons in addition to prostaglandin-depleting agents such as indomethacin.

An extensive review of radioemesis has been compiled by the Canadian Defense Research Establishment Ottawa (126). They reviewed accident cases and therapeutic radiation treatment to predict the dose response relationships for man. They have projected time to vomiting, number of emetic episodes and total time of emesis. Data are currently being harvested to evaluate the antiemetic effects in these radiotherapy patients. The United Kingdom will also evaluate the responses of radiotherapy patients in the next year.

Very recent studies by USAFSAM at AFRRI comparing emesis in dogs after cobalt-60 on neutron irradiation revealed an ED50 of 346 rads for cobalt-60 and 507 rads for fission neutrons (127). The previous study was repeated in 1980 which showed an ED50 for reactor gamma radiation of 457 rads and an ED50 for reactor neutrons of 379 rads. It is hoped that these somewhat disparate results will be resolved after further collaboration between USAFSAM and AFRRI investigators.

It is now recognized that histamine, which is released secondary to irradiation, plays a major role in the systemic manifestations of radiation injury (56-60). Histamine will induce gastric acid secretion, by which gastroenterologists diagnose certain disease states. It has also been known that widely used histamine antagonists do not block or prevent this effect of histamine on gastric acid production. Research in recent years has produced evidence that there are at least two types of receptors for histamine in the body. The sites affected by conventional "antihistamine" drugs are now designated "H1 receptors" and those in the gastric mucosa are "H2" receptors. Several new drugs block the H2 receptors of histamine and could therefore inhibit the gastric secretion that would normally ensue from histamine released in the irradiated person. What is not known is whether the blocking of H2 receptors will affect radiation-induced nausea and vomiting (114). These are effective orally, and their use in a field situation would be feasible. In relation to cancer chemotherapy, it has been found that marijuana provides protection from vomiting, and appears to be better than other anti-nausea drugs (128). There is evidence that prostaglandin inhibition of gastric secretion is not related to interference with the H2 receptor (129). Thus, the possibility that there may be agonist or synergistic effects of these newer compounds needs to be evaluated as well.

It is becoming increasingly clear that there are many effects of radiation on intestinal function that may be involved in radiation-induced diarrhea and that may also be mitigated by medical means. Experimental evidence suggests that certrain resins (cholestryramine) will bind bile acids that have been associated with diarrhea resulting from radiation injury (130). As noted previously (vide supra), sodium chromoglycate exerts an inhibitory effect on histamine-induced diarrhea (112). In addition, recent studies (131) showing a radiation-induced decrease in the integrity of intestinal tight junctions suggest that agents that increase the integrity of these junctions (132,133) may also be useful in alleviating radiation-induced diarrhea. It should also be noted that hypersecretion into the intestinal lumen associated with diarrhea may within itself trigger nausea and vomiting.

Technical Approach

In vivo and in vitro gastrointestinal preparations will be used to assess the effects of radiation on membrane permeability, transport mechanisms, and perfusion. Histochemical and electron microscopic techniques will also be used to determine the extent of radiation-induced damage to the cell system lining the GI tract after exposure to different qualities of irradiation. Noninvasive techniques using radioactive tracers will be used to quantitate the magnitude of dysfunction. Additionally, a very interesting proposal to study gastrointestinal dysfunction after 8 MeV neutron irradiation has been presented by investigators from the University of Washington.

Milestones

FY83 - Quantitation of gastrointestinal dysfunction after photon irradiations.

FY84 - Quantitation of gastrointestinal dysfunction after neutron irradiation. Determination of GI system RBE.

FY85 - Quantitation of gastrointestinal dysfunction after mixed-field irradiation.

FY86 - Quantitation of dose-reduction factors of gastrointestinal dysfunction with prophylactic agents after photon irradiation.

FY87 - Quantitation of dose-reduction factors of gastrointestinal dysfunction with prophylactic agents after mixed-field and neutron irradiation.

AFRRI will synthesize the results and combine them with symptomatic therapy of nausea, vomiting, and diarrhea to decrease the disability caused by gastrointestinal dysfunction.

RADIATION-INDUCED HEMATOPOIETIC DYSFUNCTION

Destruction of bone-marrow cells leads to marked decrease in white blood cells (leukocytes), platelets, and later of the red blood cells. The full-blown clinical picture may include anemia, bleeding, and infections as a result of radiation damage. Advances in blood-banking technology provide the prospect for replacement transfusions of red blood cells (to correct anemia), platelets (to stop bleeding) and white blood cells (to combat infectious complications). There has been significant development of technology for the freezing of red blood cells, thus allowing indefinite storage and availability in emergency situations. Progress is rapidly being made in the application of freezing technology to the preservation of white blood cells and platelets (134). Availability of ample supplies of frozen blood cell components are essential for the prompt medical support of radiation-injured soldiers.

For doses of gamma radiation below 1000 centigray, when the hematopoietic syndrome predominates, rigorous therapy can save the life of the irradiated individual (135). Red and white blood cell and platelet precursors are more sensitive to radiation. Of the readily available laboratory tests, lymphocyte

levels are of the greatest use and value as an early criterion for judging radiation injury. Early bone marrow examination may give some insight on the extent of erythropoietic damage. In further treating the patient, any procedure that would enhance white cell and platelet production would improve survival postirradiation. Procedures that can cryopreserve human leukocyte antigen (HLA), compatible white cells, and platelets, and make available these cells for transfusion during the time of stem cell recovery will increase therapeutic efficiency.

Manipulation of the stem cell offers hope of salvaging lethally irradiated soldiers. Recent studies at AFRRI in irradiated mice showed a 30-day survival of 13% in the irradiated mice without manipulation of their stem cells. Endotoxin given postirradiation increased survival to 33%. Endotoxin plus increased red cell volume, given postirradiation, improved survival to 88% (136).

Stem Cell Manipulation

Bone marrow stem cells must constantly renew themselves and also produce progeny that develop into white cells (granulocytes, monocytes, macrophages, and lymphocytes) and platelets. The primary function of these white cells is to fight infection. Since stem cells must divide continuously, they are highly sensitive to the effects of ionizing radiation. Doses between 200 and 400 centigray destroy a sufficient number of stem cells to result in decreased white blood cell (lymphocyte, granulocyte, monocyte, and macrophage) production and impaired protection against infection. What are the specific stimulatory and/or inhibitory agents that control white blood cell and red blood cell production from stem cells? How do irradiated stem cells respond to these agents? What therapeutic means could increase specific white blood cell production to the point where defense against infection is possible?

Technical Approach

Efforts will be made to increase white blood cell production, possibly at the expense of red cells until the stem cells have restored the system to acceptable balance. Both cells are derived from the same stem cell, depending on the proper stimulatory agents. Initially, hypertransfusion of packed red blood cells to polycythemic levels (to decrease endogenous red blood cell production) will be extended to other species. Eventually, white blood cell production will be controlled biochemically.

Bone Marrow Rescue

Bone marrow transplantation offers the only hope of survival to radiation casualties whose stem cells have been destroyed. Bone marrow transplantation has shown considerable promise in the treatment of aplastic anemia and refractory acute leukemia. Aplastic anemia patients receiving bone marrow transplantation from human leukocyte antigen(HLA)-compatible donors show a 30-month survival of 57%, as opposed to a 25% survival in nontransplanted patients (137). Graft failure (20-40%) is usually secondary to patient presensitization and graft rejection. These patients have usually received numerous transfusions, sensitizing them to foreign HLA antigens (138). A

variety of protocols have been developed to enable engraftment of the presensitized patient (99). The most significant complication of bone marrow transplanation is graft-versus-host disease (GVHD). The acute form of this disease is thought to be mediated by T lymphocytes present in donor cells during engraftment. The occurrence of GVHD is a major obstacle to successful bone marrow transplantation between nonrelated HLA recipients. The major requirements for successful transplantation are lack of presensitization and HLA compatibility (139).

A radiation casualty, who had received 600 centigray gamma whole-body irradiation, was successfully treated with a bone marrow transplantation from his identical twin brother (140). Because of this successful treatment of a lethally irradiated person and the tremendous progress in bone marrow transplantation for other diseases in the American medical community, a seminar was held at AFRRI in September 1980. Foremost bone marrow transplantation experts met for 2 days. They reviewed the current capabilities and made recommendations as to future directions for DOD. Eugene Cronkite, former Medical Director of Brookhaven National Laboratories, summarized the meeting (141):

> "It was clear that the civilian community utilizes bone marrow transplantation in a variety of clinical conditions. While this mode of therapy is currently used in the treatment of end stage leukemia and aplastic anemia, it has clear cut application to the treatment of irradiated casualties. While the major problems in the civilian community seem to be appropriate HLA matching and the problems with GVHD, the overall survival rate was 50% being somewhat higher in certain kinds of disease processes. This must be viewed in the context of the types of patients currently being treated. Those with end stage leukemia and aplastic anemia unresponsive (sic) to all other forms of therapy. These individuals have no other form of therapy available to them. In terms of treatment of radiation casualties, it was clear that if one had bone marrow stored at some location it could easily be given back to the individual, avoiding the problems of HLA matching and GVHD. It was also obvious that while it is impossible at this point to treat even a limited number of casualties, the scientific and technical problems of avoiding GVHD could be overcome with appropriate research and development. Such accomplishment would allow for BMT to be a highly successful procedure that would clearly benefit not only the military but the civilian biomedical community as well."

During the next 5 years, AFRRI will address the following aspects of the problem. At radiation doses of gamma above 500 centigray, nearly all bone marrow cells will be destroyed. Is the mass storage of typed bone marrow cells feasible? What are the specific causes of graft-versus-host-disease and what countermeasures are feasible? Can graft-versus-host disease be eliminated in lethally irradiated recipients receiving bone marrow grafts devoid of lymphocytes--that is, grafts composed of partially pure populations of hematopoietic stem cells? To what extent will administration of immmunostimulants affect radiation-induced immunosuppression, and will this treatment affect the incidence of late effects?

Technical Approach

Individuals exposed to a mixture of neutrons and gamma radiation, such as that found in nuclear weapons, may respond differently to the radiation exposure and also have different requirements for bone marrow transplantation therapy. A small animal model will be used to statistically validate requirements of hematopoietic resource after fission neutron-gamma irradiation.

Milestones for Hematopoietic Dysfunction

FY83 - Establish mouse model for "mixed" radiation injury and recovery.

FY84 - Establish requirements for marrow cell rescue in the "mixed" radiation mouse model.

FY85 - Establish wound and/or burn trauma model in "mixed" radiation model.

FY86 - Determine feasibility of marrow cell rescue in combined injured "mixed" radiation model.

FY87 - Determine lymphomyeloproliferative quality and response to antigenic challenge in long-term survivors.

MEDICAL AND SURGICAL TREATMENT OF COMBINED EFFECTS

Army QRR 14.4Q1 describes the military relevance of combined injury:

> "The impact of exposure of personnel to a nuclear detonation combined with other external trauma (injuries, infection, chemical and biological agents, and psychological stresses) on the ability of a man to perform his prescribed tasks is unknown. Knowledge of these factors will allow valid predictions of the combat effectiveness of troops exposed to various environments on a nuclear battlefield. Currently, personnel risk levels do not take into consideration these integrated effects, and combinations of these stresses may greatly influence establishment of personnel risk (troop safety) criteria for friendly combat personnel." (4)

The interaction of nuclear weapons effects and other battlefield trauma must be determined in order to develop diagnostic and therapeutic means to counteract the increased casualty load expected. Lack of this information could handicap medical support of casualties on a nuclear battlefield.

In addition to blast, thermal, and ionizing radiation, other factors such as chemical and biologic warfare agents and nonionizing radiation are very likely to be present in a combat environment. It is therefore of great importance to determine the impact of these agents in combination with ionizing radiation on the overall response of personnel and the effectiveness of these agents in enhancing early and late effects of ionizing radiation. Since, in a future combat environment, combined or multiple injuries will be more predominant than radiation damage alone, it is important to study the

physiologic and biochemical mechanisms involved in order to develop proper medical counteractive measures.

Injuries, benign when alone, become lethal when combined with relatively small doses of whole-body irradiation. This has produced a unique set of problems for the military surgeon. Unfortunately there are very few clinically relevant studies for guidance. The decrease in survival with combined injuries has been observed for many years in automobile accident victims. Blunt or sharp trauma by itself may cause minimal morbidity, but the addition of a small burn will often result in death. Radiation injury also greatly magnifies the problem. Experiments in animals have shown that the addition of sublethal irradiation to a burn of 20% body surface area will raise the mortality rate from 12% to 73% (142-145). Equally impressive results have been observed in pigs, rats, and guinea pigs. Estimates of incidence of combined injuries have been predicted, based on the Hiroshima and Nagasaki experiences (146) as follows:

Burn and wound and radiation injury	20%
Burn and radiation injury	40%
Mechanical and radiation injury	5%
Others	35%

Sheep exposed to a mixed neutron and gamma dose of 400 centigray and 1 hour later subjected to an abrupt overpressure suffered increased mortality from 25% to 50% (147). Messerschmidt shows that the mortality in mice rose from 26% to 90% when combined with open wounds on their backs. If the wound was immediately closed, mortality was 18% (147). Messerschmidt summarizes much of the Warsaw Pact data by stating that an open wound markedly increases the chances of infection and recommending that the wound be closed immediately (148). It should be noted that work with a mouse model at AFRRI (149,150) showed that wound trauma before irradiation raised the LD50/30 to a DRF of 1.2 and that wound trauma after radiation did not decrease the LD50/30. Dogs with experimentally infected bullet wounds at various times postirradiation showed that surgery should be performed immediately after the acute radiation insult or injury (151).

Further data suggest that open wounds greatly increase the chance of septicemia and should therefore be closed. Since most battle wounds are contaminated, either type of wound management, open or closed, is a compromise and places the patient at great risk. Guidelines based on relevant trauma conditions are required. Studies have not investigated the possibility that biochemical parameters may help to guide therapy. In addition, it would be very useful to know the total absorbed dose of radiation for which a second injury results in death.

The mechanism of irradiation injury involves two factors. First, bone marrow-derived stem cells are very sensitive to irradiation and may require more than a month to recover from a sublethal dose. During this period the patient's immune system is dangerously suppressed. It is the profound immunoparalysis that makes radiation so much worse than other forms of

combined trauma. Second, radiation injury produces portals for infection, often from the intestine, by the host endogenous bacteria (152,153).

Enhanced White Cell Production and Function

Ionizing radiation compromises the animal with respect to its natural defenses against infectious disease, indigenous gut-derived bacteria, and associated toxins. An early, nonspecific cell-mediated resistance phase plays a vital role in the first line of defense against bacterial disease. Murine models have been developed in which these defense mechanisms may be analyzed with respect to radiosensitivity, enhancement of the cell-mediated phase, and stimulation of bone marrow-derived precursors responsible for replenishing mature, function end cells necessary for ultimate recovery from the effects of radiation and the infectious process.

The major part of early resistance to infection is determined by the ability of both the circulating and the tissue granulocytes and macrophages to kill invading microorganisms and to release a variety of immunostimulatory and hematopoietic factors. Macrophages are relatively radioresistant, and survive for long periods of time in situ. It follows that their function during periods of leukopenia after radiation injury is important. Galelli and associates (154,155), using a murine model, have recently implicated the primary role of radioresistant cells in the nonspecific resistance to infection as well as the ability of this cell population to be enhanced functionally by treatment after lethal exposure. Mice treated with endotoxin after lethal irradiation resisted a virulent infection, which was only temporary unless the mice were reconstituted with fresh bone marow cells (154,155). Thus reconstitution ensured a more definitive survival against the infectious process. Complete enhancement of the resistance to infection by endotoxin is therefore mediated by the nonspecific stimulation of radioresistant cells (granulocytes and macrophages) followed by activation of and repopulation by radiosensitive bone marrow-derived progenitor cells.

Enhancement of the early resistance phase by endotoxin played a key role in consequent survival of the animal. Agents such as endotoxin have had restricted use in larger animals and man because of toxicity (156). Current work is concerned with detoxifying such agents while maintaining their therapeutic efficiency. Other such agents in current use are Corynebacterium parvum and glucan. Both are promising in terms of enhancing resistance to infection (157,158) and stimulating the hematopoietic system (159-162).

Technical Approach

The consequences of various traumas have not been well characterized. Information on responses to burn and wound (surgical) trauma in addition to placement and extent of injuries needs to be evaluated. Skin, muscle, and fracture (single and multiple) injuries needs to be evaluated. These studies should be conducted in an animal model (mice) in which statistical evaluations may be made. Once this information is obtained, injury combinations including radiation can be evaluated, and responses can be measured for the protective and therapeutic regimens used.

There are no well-characterized models for combined irradiation injury in larger animals, including models for bacterial sepsis and management. The dog is a species with sufficient blood volume to tolerate long-term studies, so it will be studied under control, surgical trauma, radiation, and combined conditions. Multiple parameters will be observed. When the control and experimental conditions are well established, additional biochemical indicators of cell injury will be investigated.

Milestones for Treatment of Combined Effects

FY83 - Develop a mouse model of wound and burn trauma with establishment of biochemical indicators alone and with cobalt-60 radiation. Develop a canine model and standard surgical methodology, with particular reference to effective radiation dose range and suitable sepsis model.

FY84 - Apply mouse model of traumas and biochemical indicators in mixed neutron-gamma exposures. Perform surgical procedures in noncontaminated wounds.

FY85 - Determine immune and myeloid repopulation dynamics in combined injury models. Perform surgical operations after radiation in noncontaminated wounds.

FY86 - Evaluate radioprotective agents in combined injury models with cobalt-60 and neutron-gamma exposures. Perform surgical operations after radiation and contaminated wounds.

FY87 - Evaluate marrow transplantation in combined injury models and cobalt-60 and neutron gamma exposure. Synethesize the results.

DOSIMETRY

The Army Qualitative Research Requirement (QRR) for nuclear effects states in Section 14.1a1:

> "Although it is recognized that the biological nuclear radiation effect is the governing casualty producing mechanism over a considerable range of yields of nuclear weapons, enough is still not known concerning the reaction of biological systems to nuclear radiation below the supralethal level. Specific organ system and cellular reactions which lead to the individual becoming a casualty are not fully understood. For example, even though bone marrow depression is the major pathological response at levels of irradiation below 1000 rad, the exacerbating effect of infection through the intestinal epithelium is unknown. Knowledge of these reactions will enable more rational and rapid development of effective measures to evaluate and reduce casualty production and treat patients." (4)

The biomedical effects of radiation have been extensively studied and are well-documented. This is particularly true of gamma radiation, and the biomedical effects of neutrons are comparable, with some exceptions. For example, it appears that neutron radiation is associated with an increased incidence of cataract formation by a factor of 10 over gamma rays. Also, there is some evidence that neutrons are less effective in producing behavioral incapacitation than are gamma photons. On the other hand, neutrons have greater biological effectiveness with respect to lethality of cancer induction. The lethal dose for 50% of persons (LD50) is still disputed. The Space Radiation Study participants considered the available data on humans and developed a dose-response relationship, fitting a normal distribution to the data yield, an LD50/60 of 286 + 25 centagray standard error of the mean, and a 95% confidence limit of 236 to 336 centigray. Similiar results were obtained with prompt radiation doses in normal dogs and primates (163). Persons dying from LD50/60 exposures die from infection within 60 days. The limiting exposure dose with maximum therapy seems to be 800-1000 rads. Bone marrow transplantation studies in patients with aplastic anemia and acute leukemia reveal that radiation damage to the lungs after bone marrow rescue may be the complicating factor in survival (163-165). The emphasis in this portion of the plan is radiation injury. It must be recognized that the effects of radiation are markedly exacerbated by non-radiation effects of nuclear weapons: burns, trauma, fractures, and fatigue (166). Present instructions for care of radiation casualties have remained essentially unchanged for the past 20 years; they consist of the administration of blood products and antibiotics, and nursing care (167).

With increased sophistication of our military systems, advanced training and specialization have become essential. It is imperative that we have medical care systems that protect, salvage, and rapidly return injured personnel to combat. Early diagnosis, protection, and treatment economize our force structure, in addition to decreasing the training and replacement requirements for personnel. An additional benefit is the impact on troop morale,

when the combatant knows that "state of the art" medical care is available. Diagnosis of radiation casualties will be based on patient history (patient was in the area of detonation), prodromal symptoms, laboratory changes, and dosimeter readings.

Army QRR 14.2a1 states:

> "The current method of determining the future combat effectiveness of troops following exposure to nuclear radiation has many shortcomings. A unit is classified in one of three broad radiation status categories based on averaged dose measurements from a few tactical dosimeters. The reliability of this method is limited by the inaccuracies associated with tactical dosimetry such as the orientation and location of personnel wearing the dosimeters, and the inaccuracy of the dosimeter itself. Additionally, the variation in human response is considerable, further limiting the usefulness of this method. In short, unreliable casualty estimates and an unaffordably inefficient use of remaining personnel assets." (4)

Current research in battlefield dosimetry has two recognized needs: triage of exposed troops and the breakdown of dose for medical treatment. Triage is a comparatively straightforward concept, commonly defined as the separation of casualties into three distinct groups: (a) those requiring no medical attention because the absorbed dose is small enough to create no significant somatic response, (b) those requiring only supportive care because the dose is too great to permit recovery after available medical treatment, and (c) those requiring definitive medical attention because the casualty has a significant chance of recovery. The vagueness of the lines separating the three categories is a matter of extensive debate; the lines will depend on conditions such as treatment environment, dose distribution, any compounding injury, and individual response to radiation.

The last characteristic is the spark of interest in biologic dosimetry. The only sure thing a soldier carries is himself. In addition, the biological dosimeter may eliminate part of the most uncertain steps in dosimetry: the relation of dose received by the dosimeter to dose received by the subject. Placement, shielding, and dose distribution will always be significant problems, but the elimination of several magnitudes of doubt can be achieved by using the body itself as a dosimeter.

BIOLOGIC DOSIMETRY

Army QRR 14.2b states the following specific military requirements:

> "(1) Identification of biochemical parameters that will provide an early, accurate indication of the degree of radiation injury sustained.
>
> (2) A clinical test giving absorbed radiation doses based on those biochemical parameters and suitable for use in field medical facilities.

(3) A method for use by medical personnel in the field which quickly identifies supralethally irradiated personnel to the triage surgeon." (4)

Most of the current research in biological dosimetry is being conducted at the Armed Forces Radiobiology Research Institute (AFFRI). This effort may be divided into four categories: blood studies, waste product studies, electron paramagnetic resonance (EPR) techniques, and Fluorescence-Activated Cell Sorting (FACS). At present, most of these dosimetry methods are in the stage of developing usable dose-response curves; conversion to functional dosimetry has not yet begun.

Blood Studies

The serum levels of many compounds change drastically upon irradiation. Concentrations of various metals, glycoproteins, and myoglobin are being investigated at AFRRI. The in vitro and in vivo techniques now used are certainly beyond the scope of even the most sophisticated battlefield clinics, but the biological significance of these changed serum concentrations may prove to be an invaluable marker of radiation damage. The primary method for determining serum trace metals is atomic absorption spectrophotometry. Changes in magnesium, copper, zinc, and iron levels are the most notable. In studies at AFRRI, levels of serum iron increased from 50 to 500 centigray in a linear fashion. However, at radiation exposures greater than 500 centigray, no additional increase was seen (168). It may be necessary for more than one biochemical assay to be used to describe the casualty's radiation burden (10-1200 centigray).

Postirradiation increases in serum levels of glycoproteins and protein-bound carbohydrates such as sialic acid are being studied as possible markers. These radiation-induced changes are particularly significant because evidence is accumulating that many of these proteins have immunoregulatory properties. Methods of detection are still overly sophisticated and time-consuming. Techniques vary from radial immunodiffusion and nephelometry for glycoproteins to chemical determination of sialic acid and neutral hexoses. The trauma induced by radiation may also cause significant amounts of myglobin, an iron-containing protein, to be released by skeletal muscle. Immunodiffusion and radioimmunoassays have become very sensitive indicators of serum myoglobin, but a viable dose-response curve has yet to be established. As with other trauma-induced biochemical changes, it is often difficult to differentiate between radiation changes and other injury changes.

Waste Product Studies

Waste product studies are an adjunct to blood studies, and they are more easily adaptable to useful dosimetry. They comprise a segment of research devoted to the increased output of several possible radiation markers in urine. The early interest in deoxyribonucleic acid (DNA) breakdown products has not given useful results. The early enthusiasm for deoxycitidine and beta aminoisobutyric acid (BAIBA) has diminished because of the tremendous variation among species (169,170).

Increased urine levels of zinc and sialic acid postirradiation have been studied. Other possible biological markers in urine are the histamine released by mast cells postirradiation and various polyamines (spermine, spermidine, putrescine). Although the blood histamine is soon dissipated via histaminase, the kidneys filter about 1 percent to urine, in levels that are easily quantified. The primary method for detecting histamines is fluorometry and for sialic acid it is colorimetry, relatively simple processes with significant promise as battlefield methods. Lipid peroxides, which may decompose to short-chain hydrocarbons, are produced in the body upon irradiation.

Preliminary experiments at AFRRI have shown that, indeed, ionizing radiation causes damage to cellular membranes, resulting in the production of volatile hydrocarbons such as pentane. Current studies are aimed at measuring pentane and other volatile hydrocarbons in the breath of animals exposed to ionizing radiation, to determine if those substances can be used as biologic indicators of radiation injury.

Experimental evidence in our laboratory has also shown that histamine and prostaglandins are released into the urine of irradiated rats and that this release may be dose-dependent. Therefore, the possibility of their use as indicators of radiation injury is of great importance and should be investigated (171).

Milestones for Blood Studies and Waste Production Studies

FY83 - Comprehensive reassessment of new techniques for measuring biochemical indicators of radiation injury in physiologic fluids.

FY84 - Systematic analysis of selected serum and urinary constituents for use as a biological dosimeter with techniques that have the greatest promise.

FY85 - Simplification or modification, if necessary, of biochemical assay techniques of those constituents having the best dose-response characteristics.

FY86 - Validation of techniques using different qualities of radiation.

FY87 - Automation of the developed assay technique for possible use in the battlefield.

Electron Paramagnetic Resonance (EPR) Techniques

Free radicals that can be observed and quantitated by EPR are produced in bones and teeth when these calcified tissues are exposed to ionizing radiation. The EPR spectra taken from these samples are complex, suggesting that a number of different radicals are produced. Although some radicals may exist in the organic phase of these tissues, probably most reside in their crystalline matrices of hydroxyapatite as electron traps. Some of these lattice defects are sterile in vitro for only hours and days whereas the majority of them have a half life of 10^8 years (172). Their stability in vivo is unclear, with the only reported study to date showing no decrement in the major signal for up to 3 weeks after irradiation.

The different stabilities of the radicals produced may offer a means to assess time of exposure as well as total integrated dose. Measurement of the ratio of intensities of the stable to unstable peaks could yield an estimated exposure date within a time of almost 2 weeks postirradiation. The stable signal is proportional to the cumulative dose and shows a near linear dose-response relationship from 200 to 10^7 rads where saturation occurs (173). Doses of less than 200 rads seem to display nonlinear behavior. The sensitivity of this technique has been developed here at AFRRI so that detection in the tens of rads is now possible with as little as 50 mg of bone or tooth sample. A method of measuring these signals noninvasively from a digit is now under collaborative investigation with RADIAC.

Fingernails and toenails also display differences in their EPR spectra between control and irradiated samples. However, the dose-response relationship of this tissue seems to be too low to offer much promise as a viable dosimetric method.

Presence of natural signals, variability in the hydroxyapatite of the calcified tissue, and stability to the field environment remain as obstacles in developing this technique, but EPR studies are an exciting possibility.

Milestones for EPR

FY83 - Development of dose-response curves after gamma radiation by EPR on in vitro specimens.

FY84 - Development of dose-response curves by EPR on in vitro specimens after neutron irradiation.

FY85 - Extension of dose-response curves by EPR to blind the interpretation of different qualities of radiation.

FY86 - Development of in vivo dose-response data by EPR.

FY87 - Prototype development of EPR spectroscopy with RADIAC.

Fluorescence-Activated Cell Sorting (FACS-II)

The FACS-II separates cells on the basis of fluorescence and size (174). This is accomplished by confining cells of interest to the center of a liquid stream and causing them to pass one at a time through the focused beam of a laser. The signals are processed by the instrument, which then determines whether or not each cell meets certain operator-selected criteria. If it does, the cell is identified as one that should be separated from the other cells within the stream, and it is directed into an appropriate container.

Recently the FACS-II was upgraded from a one-laser system to a dual laser system. As such, the potential exists to measure simultaneously eight different cellular parameters on individual cells as they pass (500 per sec) through the two laser beams. Some cellular parameters that can be measured are DNA content, RNA content, plasma membrane receptors, surface antigens, hydrocarbon viscosity, surface mobility, and surface area. Physical properties of cells such as size, shape, and refractive index as well as nucleus-to-cytoplasm ratio can be determined with this instrument. The

recent work of Chaudhur, using peripheral reticulocytes as a biological dosimeter, suggests that this is a potentially powerful technique (175).

The distribution of physical, structural, and functional properties of individual cells within irradiated and nonirradiated heterogeneous cell populations can be assessed and coupled in a logical manner with irradiation dose and/or quality. Data derived from this type of experimentation would be of immense value in the design of biological dosimeters.

Milestones for FACS-II

FY81 - Installation of a stand-alone on-line computer that would acquire simultaneously four to eight analog parameters. (Using this system, 100,000 cells could be analyzed in less than 2 minutes.)

FY83-84 - Development of software for: (a) the programmed search for significant correlations among signals in multiparameter space, and (b) the automatic progression within a given sample.

FY85 - Correlation of changes in cellular parameters and proliferative potential of irradiated lymphatic and hematopoietic cells with radiation quantity and quality.

FY86 - Assessment of the results of this approach to dosimetry, and comparison with results obtained using other biological and physical dosimeters.

PHYSICAL DOSIMETRY

An improved dosimetric system that would allow characterization of the amount and quality of radiation received by an organism or a particular cell type in an experimental radiation field is of great significance, and it should be developed. This dosimetric system will likely be applicable to mono-and polyenergetic neutron, gamma, and mixed neutron-gamma radiation fields as found in a future combat environment. Survival is related to bone marrow injury, so it is important to provide bone marrow dose plus the free-in-air and midline doses. In addition correlation between the tactical and personal dosimeter and the laboratory techniques using a human phantom should be made (176). Since the tactical dosimeter will be used for command decisions and the personal dosimeter for medical decisions, it is important for the commander and medic to know what the dosimeter reading means.

The mixed fields produced in the reactor are varied in spectral component and in distribution. Major differences occur not only from array to array but also between different location in the same array. Because these spectra do not always match the requirements of the investigator and because there is often gross variance in biological response due to radiation energy, the investigator must be given the radiation spectra that concern his experiment. Characterization of the fields is important not only from the investigator's point of view but is critical for the dosimetrist in the design of the experiment. The option to tailor the field to best meet the needs of the investigator is essential.

Acquisition of this ability to map the reactor fields was begun under SAI contract. The first contract, let in 1979, produced spectral maps for specific locations in five different experimental arrays. The spectra were drawn via activation techniques coupled with Monte Carlo calculations. One-dimensional and three-dimensional codes were ANISN and MORSE, respectively. ANISN was used when possible in order to keep the cost minimal and where MORSE gave unacceptable results due to the limiting assumptions. The results verified the use of ANISN except where fields had been grossly perturbed by significant masses of hydrogenous material. For these perturbed fields, a modified version of the three-dimensional MORSE code gave best results.

A second contract has been proposed. This contract would load the computerized results of the first contract into the AFRRI computer system as well as the AFRRI-tailored ANISN code. This would enable the dosimetrist to transport the spectrum through the experimental array and calculate the spectra at the points of interest. Note that this one-dimensional code remains valid only for arrays where the field is not significantly affected by large, hydrogenous objects, such as animals, in the exposure room.

The three-dimensional modified MORSE code might be the object of a third contract. Given enough specific data, the code could be used to generate spectra at depth. Verification of these spectral changes would require considerable effort. When, after required modification, the AFRRI code proved empirically valid (microdosimetry?), phantom/animal modeling could be accomplished. Dose distribution studies, including bone marrow dose and activation dose, could begin. Arrays could be optimized efficiently to provide the requested field and sulfur or ionization chamber results could be easily interpreted as a field check. This would be ideal from a biological standpoint. The investigator could be provided with a field design that meets his/her specific requirements, this field could be presented in quantitative form, and doses could be verified with current or microdosimetric techniques.

The entire process as outlined in the three contract form above might be accomplished in-house given the codes and cross section data. This is a considerable effort. Even if the current computerized codes and cross-section data were purchased from SAI, the task of getting the codes on line, validating them, and developing a usable system constitutes man-years of work. Only then can animal modeling begin.

An additional benefit of mapping these fields on the computer is the ability to compare spectra in the reactor to those of concern on the battlefield. Knowing the spectra of interest in each experiment is of as much military benefit as scientific benefit. The scientist deals with spectra to compare effects between experiments, and the military would apply the scientific results to the tactical environmental.

Extrapolation to man has always been a task of extreme uncertainty. The ability to regularly quote spectra and fluence at points of interest within an experimental animal or within a petri dish make the extrapolation an order of magnitude more valid. This represents the solution to the experimenter's physical problem: Has the biological mechanism/organism received a dose

comparable to what that would be received in man? The problem of biological system effects would still exist, but at least the biologists' experimental efforts could be meaningfully compared.

Although the mixed field produced in the reactor is the one most in need of this capability, the other radiation fields produced at AFRRI could benefit in the same manner from this computerization. Although the gamma and electron fields are more readily comparable, radiation biology still suffers from the same spectrum and fluence distortion effects whenever results are compared or analyzed. Rads (0.511 MeV gamma) may be as biologically different from rads (1.25 MeV gamma) as rads (1 MeV neutron) for a given mechanism. Failure to quote spectrum and fluence for an experiment may constitute the source of significant error.

The utility of this effort may not be readily apparent. Given AFRRI's current abilities, dose quotes and neutron-to-gamma ratios are readily available. However adequate today, the time is soon coming when biological systems investigation will require more specific information. Radiological physicists are only beginning to use Monte Carlo techniques to radiation fields. Further applying these methods to biologic investigation would put AFRRI in the forefront of the field. To do less would shackle biologists needlessly, at first only from the requirements imposed by good sciences, and eventually from their colleagues.

Microdosimetry

Energy deposition in matter due to radiation is stochastic. Current radiation measurement methods at AFRRI give absorbed dose, the result of a probabilistic process. Given the current trend toward more demanding dosimetry from the biological investigators, the need for more detailed knowledge of the actual energy deposited at sites on the order of microns and nanometers will become pressing simply because these sites are more biologically relevant. Spectral information as well as energy deposition will be required. The answers to these important questions are studies via microdosimetry.

Approaches to microdosimetry vary. Because it is a relatively new field, no one technique stands out as standard. Usually a combination of computational and measurement methods is used to provide a measure of the distributions in linear energy density, or spectral and fluence knowledge on the molecular scale. Different measurement methods include wall-less proportional counters, thermoluminescent dosimeters, track etching, cytogenetics, and cloud chamber techniques. Certainly the method would have to match the information required. Although none are out of bounds with respect to need, proportional counting and TLD's are more within the realm of existing capabilities.

Varied uses of this information exist. Besides the biologically important specific energy distribution within the mechanism, one would be able to verify and correct for wall effects seen by our current ionization chambers and make necessary correction from TLD to tissue or bone marrow dose. We would also be able to verify computer modeling techniques via actual measurement.

Technical Approach

AFRRI will investigate different calculational techniques for depth-dose determination. Various computerized models will be developed with respect to neutron and gamma depth-dose distribution to provide midline tissue dose and bone marrow dose. Dosimetric readings will be correlated with free-in-air dose, midline dose, and bone marrow dose calculated from phantom studies for different qualities of radiation. From this determination, recommendation will be made on the most effective wearing position of personal dosimeters.

COMPUTER MODELING OF AFRRI REACTOR

Requirement	Possible Solution
Quote spectra free in air	Possible via SAI contract report for selected places in selected arrays. Varying location or array would require interpolation or implementation transport capability by installing ANISN transport code on AFRRI computer system.
Measure spectra free in air	Activation analysis coupled with a computerized unfolding code such as SAND-II. Proportional counting techniques may suffice for gamma energies and fluences.
Quote spectra at depth	Adjoint techniques using MORSE as well as ANISN codes
Measure spectra at depth	In addition to air spectral measurement techniques, microdosimetry may be used to measure doses.
Field tailoring	Computerized standard parameters, used to give fields of interest, may be coupled with known battlefield spectra data.

Milestones for Physical Dosimetry

FY83 - Implement routine determination of bone marrow dose.

FY84-85 - Determine bone marrow dose for gamma, mixed field, and high-energy neutrons, and compare to tactical field instruments. Develop microdosimetric techniques.

FY86 - Perform whole-body irradiation and compare tactical dosimeter readings in differing wearing positions.

FY87 - Compare results of physical dosimetry and results of biological dosimetry, and decide which should receive the greatest effort.

EFFECTS OF FAST NEUTRONS

BIOMEDICAL EFFECTS

The Army QRR 13.1a5 identifies this problem as follows:

> "Current incapacitation dose levels are based on the assumption that equal tissue doses of neutron and gamma radiation cause equivalent biological damage. Experimental results on monkeys and pigs have indicated that the effectiveness of fission-spectrum neutrons, relative to fission-spectrum gamma rays, for degrading task performance is less than unity. Further definition of the value for the RBE, to include task dependency, if any, is required so that casualty criteria may be more accurately determined. In addition, to design tactical dosimeters which will accurately weight the neutron dose, the neutron RBE must be determined."

The relative biological effectiveness (RBE) of neutrons has been a hotly contested scientific question (177,178). Several investigators have written disclaimers to the conclusions of the BEIR III report (179,180). More importantly, the data derived by Auxier (the T65D dose) (181) has recently been questioned (182). This article suggests that most of the cancers came from low-LET gamma photons. This assertion is based on the unpublished results of Loewe and Mendelsohn of Lawrence Livermore and complemented by Kerr of Oak Ridge, who is performing similar work. Until the question of RBE based on Hiroshima is resolved, a greater reliance on experimental results must be used. As early as 1939, neutron therapy was used in the treatment of cancer by Stone and Larkin when the Lawrence cyclotron at Berkeley, California, became available for medical use (183-185). During a 5-year period, 226 patients were treated. It was concluded at the end of this study that fast neutrons could eradicate cancer, but the late effects on normal tissue were more severe than expected, in comparison to the early effects seen in the same patients. Later, when Stone's results were evaluated (185), the data indicated that the California patients had been overexposed due to certain radiobiological factors unknown at the time. These factors include the increase in RBE as the size of the neutron fraction decreases (186), and the little importance then attributed to previous X-ray therapy of the patients before neutron therapy (187).

Radiobiological experiments with the Berkeley cyclotron at that time showed that neutrons were more effective than X rays by a factor as great as 11 when end points were studied, such as leukopenia in whole-body irradiated rats, tumor growth after in vivo irradiation in tumor tissue, or lethal effects on bean roots (188,189). Broerse, Barendsen, and Van Kersen showed that RBE will increase with decreasing neutron energy (190). This was confirmed on mouse hematopoietic stem cells by Carsten, Bond, and Thompson, 1976, by using six different monoenergetic neutron beams and two fission spectra (191). The RBE of monoenergetic fast neutrons for marrow stem cell depression does not appear to vary appreciably over the range of 0.43 to 1.8 MeV. However, for 5.7-MeV and 13.4-MeV neutrons, a drop in RBE was noted. The lack of dose-rate effect for stem cell inactivation is well documented for gamma rays below total doses of 300 rad. Higher total doses have revealed such an effect (192-196).

Relatively few radiation studies have been carried out with human bone marrow cells. Under identical culture conditions, human bone marrow cells were found to be more radiosensitive to gamma rays (D_o = 85.2 ± 4.5 rad) than were mouse cells (D_o = 138.7 ± 4.8 rad) based on colony formation in agar diffusion chambers (195). Mouse cells irradiated in chambers and in situ (D_o = 137 ± 2.7) did not differ in their radiosensitivity. Boyum et al. (196) have determined the RBE values for human bone marrow cells using two different culture techniques. Neutrons with mean energy of 0.44 MeV gave the highest RBE numbers (3.7 to 4.5), whereas 15-MeV neutrons were least effective, with RBE's between 1.4 and 1.6. Fission neutrons and 6-MeV neutrons gave intermediate values. Relatively high D_o values (300 centigray) were found for the inactivation of lymphocytes as measured with diffusion chambers. These are higher than those found by transforming results from chromosomal aberration studies to survival fractions. However, they are in line with studies on interphase death (196) and transformation capability of lymphocytes by PHA stimulation (196-198) after irradiation.

Mortality Studies of Animals After Neutron Exposures

The values for RBE of neutrons generally observed in mortality studies are lower than those in experiments involving single cell inactivation. As was the case with measurements of marrow cell sensitivity to neutrons, most studies of mortality have been done with mice, although a relatively large number of other animals have been included. Death after doses in the range of LD50/30 are characterized by the bone marrow syndrome. Of 20 cited studies in the paper of Otto and Pfeiffer (198), the RBE values vary from 1.05 to 7.0, of which 13 are below 2.00. The discrepancies among the reported values must be explained by variations in neutron energy; by differences and uncertainties in neutron dosimetry; by differences in animal strain, sex, and age; by differences in animal maintenance and exposure conditions; and by differences in type, energy, and dose rate of reference radiation. The RBE's of fission neutrons for intestinal and hemopoietic deaths for guinea pigs, rabbits, sheep, and goats have been obtained (198-202). A summary of the results is given in Figure 1. Species variations are considerable, with the larger animals having the lower RBE values. On several occasions, the neutron-irradiated animals tended to die earlier than those irradiated with X rays or y rays, and the average survival time after neutron irradiation increased with neutron energy (203). However, this is not a consistent finding (204). Taking into account the shielding factors for neutrons and X rays in goats, an RBE value of 3 was calculated for hematological tissue damage (202). Broerse (204) found an RBE of approximately 2 for the occurrence of the bone marrow syndrome in rhesus monkeys by comparing X rays and fission neutrons with a mean energy of 1 MeV.

Species	Weight Range (kg)	RBE	Reference
Goats	60-95	0.75 ± 0.07	83
Sheep	15-30	0.79 ± 0.12	84
Rabbits	2-4	2.43 ± 0.12	85
Guinea pigs	0.5-1.0	2.00 ± 82	

Figure 1. Relative biological effectiveness of fission neutrons for intestinal and hemopoietic deaths (from Batchelor, ref. 201)

A current study being conducted by AFRRI and George Washington University has evaluated the comparison of cobalt-60 irradiation with 15-MeV neutrons (205,206). The initial group of animals participated in a variety of studies, and yielded a wealth of information on the effects of 15-MeV neutrons on lung, heart, brain, spinal cord, and liver (207-211). A number of salient conclusions were made from these studies. Because the dome of the liver was in the hemithorax radiation port, hepatic necrosis caused a high mortality among the neutron-irradiated dogs (207). All dogs showed significant cardiovascular effects from the radiation. There were intimal fibrosis, medial thickening and calcification, disruption and duplication of the elastic lamina, and formation of atheromatous plaque. Acute changes showed hemorrhage, necrosis, fibrosis, and vascular damage. All of these changes were significantly worse in the neutron-irradiated dogs (209,211). Similar microvascular derangements were seen in skeletal muscle, pulmonary parenchyma (the largest vascular bed), central nervous system, and spinal cord (209-213). Regardless of the methodology of computing RBE in the lung, the values obtained were always 4-6 (213). Other aspects of this study that are of possible significance to the military are the 15% incidence of cancer in 15-MeV irradiated animals with very short latency periods and the significant late tissue effects of neutrons, implying no repair phenomenon (214).

DECREMENT OF PERFORMANCE

The effectiveness of neutrons in producing performance decrement and behavioral incapacitation has been assessed, using the miniature pig and primates in studies with both fission spectrum and high-energy neutrons. The relative effectiveness of a fission spectrum neutron field was compared to that of a gamma field, in producing performance decrement in the miniature pig after whole-body radiations delivered at a rate of 2000 rads per minute. The incident neutron-to-gamma ratio for the neutron field was approximately 10. The neutron-to-gamma ratio for the gamma photon field was 0.08. Both these fields were obtained by shielding the AFRRI TRIGA Reactor with either water to thermalize the neutrons or lead to absorb gamma photons. The median effective dose for producing early performance decrement in both these fields was determined for the miniature pig performing a shuttle-box avoidance task. Comparison of the median effective doses for the miniature pig in these two fields indicates that the relative biological effectiveness (RBE) of the neutron field was 0.23 (215).

The same experimental configuration was used to evaluate the relative effectiveness of fission spectrum neutrons in producing early transient incapacitation in the monkey performing the visual discrimination task. In this study, the relative effectiveness for the neutron field was calculated to be 0.68 for primates irradiated with whole-body radiations at a dose rate of 2000 rads per minute (216).

In an effort to evaluate the effectiveness of high-energy neutrons in producing behavioral incapacitation, a study was conducted in which the primates performed the visual discrimination task after one of the following head-only irradiations: a neutron beam from the Naval Research Laboratory's cyclotron or a bremsstrahlung beam from the AFRRI linear accelerator, matched for dose rate and depth dose distribution. The size of the available cyclotron beam prevented replication of the whole-body exposure given the fission spectrum animals, but it produced very similar RBE's for incapacitation (217). The RBE calculated for high-energy neutrons was 0.56. In the study of high-energy neutron effectiveness, survival and emesis data were also taken for each of the primates irradiated in both the neutron and photon fields.

These data point up the complexity of the radiation effects and the importance of specifying end points when referring to relative biological effectiveness of neutrons, since the same neutron field, which was less effective in incapacitating animals, was found to be more effective in producing lethality in these same animals. The RBE for mortality for this study was calculated to be 1.34. Likewise, the photon field, which was more effective in incapacitating visual discrimination performance, was less effective in producing emesis, since no head-only photon-irradiated animal vomited and some subjects vomited at every dose level among the neutron-irradiated groups. These studies strongly suggest that neutrons are less effective in producing behavioral incapacitation than are gamma photons, because this finding has been replicated in two species with two different behavioral tasks for both whole-body and head-only irradiations.

Further evidence to support this point can be obtained from the large dose-response studies undertaken with primates performing a visual discrimination task. These dose-response studies have been completed for mixed-spectrum radiation fields having neutron-to-gamma ratios of 0.4 and 3.0, respectively. The median effective dose to produce incapacitation in the 0.4 field was approximately 1700 rads (218). The median effective dose required to produce incapacitation in primates performing the same behavioral task in the 3.0 field was approximately 2800 rads (219). Again, these observations contrast with those concerning survival after irradiation. A dose-for-dose comparison of the neutron-rich and gamma-rich fields indicates that the neutron-irradiated animals survive for significantly shorter periods of time after irradiation. In addition, recent data on the rat suggest that the effectiveness of neutrons and of gamma photons for producing performance decrement may not be equivalent.

Since the rat is an important experimental model used in many screening and mechanism studies, a research objective for AFRRI over the next 5 years will be to establish dose-response curves for the rat in a fission spectrum neutron and in a gamma photon, in order to calculate the relative effectiveness of these fields in producing behavioral incapacitation.

Milestones for Decrement of Performance

FY83-84 - Establish the dose-response relationship for incapacitation and performance decrement for gamma photons (cobalt-60).

FY85-86 - Establish the dose-response relationship for incapacitation and performance decrement for fission neutrons.

FY87 - Determine the relative effectiveness for these three types of radiation and integrate these data into the Comprehensive Personnel Nuclear Weapons Effects Model.

ENHANCED RADIATION

The Navy Nuclear Weapons Effects Program Technical Needs state:

> "The use of ER weapons by our own forces in defense or by an enemy force against us lessens the danger to a ship or aircraft from blast and thermal radiation but greatly intensifies the danger to the crew from nuclear radiation (specifically high energy neutrons)." (220)

This requirement specifically requested biological data on 6- to 12-MeV neutrons. As noted earlier, relative biological effectiveness, based on lethality of in vitro systems, reaches a maximum at about 300-400 KeV (193-195). Experiments with very high LET irradiation in support of the space program have showed increased neoplastic change (221). The uncertainties regarding this question must be resolved.

As previously mentioned, in the spring of 1982, AFRRI will convene a symposium on Biologic Effects of High-Energy Neutrons. This 3-day seminar will provide a review of biological data on neutrons by the most outstanding international neutron radiobiologists; a review of the radiation environment of different weapons (neutron/gamma ratio and energy spectrum) by DNA physicists; and recommendations on what questions must be answered and whether by in-house research or by contract. No programs are planned until after the conference. Programs from this conference will form the basis for the future and will be reflected in next year's version of the 5-year plan.

BIOCHEMISTRY

Research is in progress in the Biochemistry Department at AFRRI to find ways for the more effective penetration of therapeutic drugs, such as antidotes of anticholinesterases, through the blood-brain barrier into the brain. Preliminary experiments with commercially available anticholinesterases as probes have indicated that 10-min exposures to low-power density nonthermal pulsed microwave irradiation (10 mW/cm^2) would cause reversible opening of the blood-brain barrier (72). Continuation of these experiments could, for the first time, offer a technique for more effective introduction into the brain of radioprotectants that normally do not penetrate the blood-brain barrier (97).

The use of FITC coupled monoclonal antibody and flow cytometry will in the near future make HLA and in general tissue typing a common clinical procedure as blood group typing. Further, it is certain that military medicine, especially those aspects dealing with radiation casualties generated by the use of theater nuclear weapons, will make especially large demands on the monoclonal antibody technique.

The production of monoclonal antibodies will be accomplished by hybridizing lymphoid cells from an appropriately immunized donor with cells from a myeloma adapted to grow in culture. The clonal hybrid cell line producing the desired antibody is then isolated. Each hybrid clone produces a single antigenic determinant. Thus, the hybridization technique allows the standardization of large-scale production and the dissemination of specific antibody reagents, many of which could be obtained only (if at all) by elaborate absorptions of small amounts of complex antiserum. This technique could lead to the rapid separation, typing, and collection of stem cells and other blood products.

Advanced techniques and instrumentation are being used in studies to determine the radiation-induced changes in cellular membranes and in macromolecular constituents of the living cell, both in cell cultures and in the irradiated animal, and to identify the sequence of biochemical events leading to radiation-induced injury in these cell constituents.

Special emphasis will be placed on the elucidation of the biochemical mechanisms responsible for early transient incapacitation (ETI) and for histamine release during this phenomenon. A subhuman primate model that permits aspiration of cerebrospinal fluid in the awake, nonrestricted animal has been developed and will be used in the studies of ETI and of radiation-induced damage to the brain.

Elucidation of the biochemical mechanisms underlying radiation-induced injury, repair, and protection from the ionizing radiation will permit the use and development of appropriate drugs that are more effective in counteracting this damage and in protecting the organism from the deleterious effects of radiation.

Studies on the mechanisms of radiation-induced biochemical changes and the physiological effects of radiation-induced changes in serum and tissue components will provide a basis for determining the most efficient biologic dosimeters. Studies on radiation-induced immunosuppression due to partial-body and whole-body irradiation will provide a basis for the development of treatment regimes (immunostimulants, dietary factors) that can be used alone or in combination with traditional sulphydryl-containing radioprotectants.

EXPERIMENTAL HEMATOLOGY

Extensive use has been made of a murine system for screening agents and procedures, that will give insight into the basic control mechanisms for proliferation and differentiation of pluripotent stem cells and their committed progeny. This technology base is then extended, where applicable, to the canine model. Assay procedures will allow further insight into hemopoietic cell populations of the canine system and into their response to agents that stress the hemopoietic system.

Analysis of stem cell physiology has been greatly enhanced by the adoption of two specific techniques in the hemopoietic stem cell assay: (a) the osmotic "minipump," which allows continuous release of soluble chemicals or agents into the blood stream for up to 7 days, and (b) use of bromodeoxyuridine as a conditionally lethal marker, which is incorporated into the DNA of replicating cells.

Use of this technique has recently been demonstrated (222), and its applicability will allow greater insight into the mechanism that controls stem cell cycling and differentiation into committed progenitor cells. It is currently being used to investigate the response of the stem cell population to endotoxin and sublethal irradiation.

Use of new assay procedures will permit us greater insight into the response of the canine hemopoietic system to radiation and to specific agents with potential for enhancing the recovery process. These new assay procedures will allow the measurement of both erythroid and granulocyte progenitors as well as those progenitor cells responsible for establishing the microenvironment that induces hematopoiesis. Additional assay procedures will also involve the use of the osmotic "minipump" to deliver stimulatory agents such as attenuated endotoxin at continuous low-dose rates. This may allow greater hematopoietic stimulation with less toxicity.

It is necessary to determine the efficacy of elutriating canine marrow cell populations into hemopoietic subpopulations with the potential of transfusion and repopulation. Fractions of cells must be assayed for content of progenitor cells and lymphocyte subpopulations with the potential of initiating graft-versus-host disease.

Mutant mice will be developed that have variable resistance to endotoxin as well as sensitivity to bacteria that are specific to their normal strains. This will allow investigation of the mechanisms of resistance and sensitivity to radiation-induced sepsis. There is a great need to develop a canine model for sepsis, to be used in conjunction with irradiation, wounding, and surgical

combined-injury models. This will also allow verification of the mechanistic data gained in murine systems, which will determine the reliability of extrapolating data from the canine model to the human response to radiation.

Basic studies in lymphocyte and macrophage physiology and the response to irradiation are necessary. The rationales for this are that the monocyte-macrophage is a primary cell in early nonspecific cellular response to radiation sepsis. Lymphocyte functioning to antigen challenge before, during, and after radiation may have a direct bearing on survival quantity and quality. Macrophages are also involved in the release of monokines (stimulatory molecules), which affect other cells in their response to infection. Lymphocytes are involved in the production of lymphokines and inhibitory substances that maintain host capacities to defend against infectious challenges. Murine models will be investigated using irradiation and skin wounding as two initial parameters.

RADIATION SCIENCES

Research into effects of ionizing radiation on red cell membranes and lymphocyte membranes using electron paramagnetic resonance (EPR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy will continue. The effects of ionizing radiation on high-energy phosphate metabolism in the perfused heart will be studied with NMR techniques. Characterization and quantification of radiation damage to lymphocyte membranes using nanosecond flourescence and EPR spin-labels will be done. An attempt to isolate, characterize, and quantify the free radical agents of radiation damage to biological tissue will continue. Single photon emission computed tomography will be used to characterize and quantify radiation injury to multiple organ systems, including the cardiovascular, pulmonary, central nervous, and gastrointestinal.

The production of monoclonal antibodies become a reality with the production of somatic cell hybridization in 1975 (223). Antibody-producing B lymphocytes have been fused with myeloma cells which are then able to produce highly specific monoclonal antibodies. Several promising avenues of investigation are already possible: antibodies to human antigens, immunologic dissection of the body, and antibodies to pathogens (224). Among these avenues would be differentiation antigens, cell-surface antigens (which may be polymorphic), onco/fetal antigens, antigenic determinants on secreted cell products and their receptors, and anti-pathogen antibodies.

These agents are potentially useful for not only investigative and diagnostic uses, but also for a variety of possible therapeutic schemes. Anti-lymphocyte antibodies hold the possibility of modulating the immune response postirradiation, potentially allowing allograft bone marrow transplantation (225). Labeling monoclones with a variety of radionuclides will allow elegant investigations of radiation injuries using noninvasive techniques of nuclear medicine.

Until recently the effect of radiation on epithelial tissue has been studied mainly in the whole animal or in isolated organs. It is now within the capability of the Department of Physiology at AFRRI to grow epithelial cells isolated from the intestine or the kidney. These cells can be grown in culture on porous supports, thus enabling investigation of transport mechanisms for ions, amino acids, sugar, and water through a monolayer of homogeneous epithelial cells. It is also possible at this time to isolate the epithelial plasma membranes from either the mucosal side or the serosal side in the form of closed vesicles, which also allow transport studies in a pure, isolated, cell-free preparation of either of the sides of the epithelial cell. The Department will be using these techniques to investigate the effects of radiation on the primary and secondary active transport systems in epithelia, in addition to the transport through both isolated membrane portions.

In a second major effort, the Department now has the personnel and capability of using electrophysiological techniques to study the transport mechanisms across isolated segments of intestine. These techniques include voltage clamp technology, which allows the measurement of short-circuit current and ionic fluxes in the isolated intestine. In addition, the ionic transport mechanisms in the gastrointestinal epithelia can be followed, using ion-sensitive microelectrodes in combination with conventional electrophysiological techniques. These techniques allow the monitoring of sodium, potassium, and choloride fluxes across mucosal and serosal components of the intestine. In addition, the possibility of using calcium-sensitive microelectrodes, in combination with other techniques, allows the monitoring of intracellular calcium in the response of intestinal epithelia to secretatogues. This technique will also be used in combination with standard electrophysiological techniques to determine changes in ionic permeability, correlated with release of calcium from intracellular stores. The techniques represent new instrumentation for measuring intracellular and extracellular ion activities of primary ionic constituents of the external and internal cellular environment. The techniques will allow investigation of radiation's effect directly on the primary and secondary active ion-transport mechanisms in the isolated intestine.

The first studies to directly record intracellular potential and conductance changes from macrophages were carried out by AFRRI personnel. These pioneer studies, characterizing the membrane properties of the macrophage, are continuing. Recently it has been possible to voltage clamp macrophages for the first time, using a recently developed single-electrode clamp. Electrophysiological approaches include the application of voltage clamp techniques to the study of macrophage membrane bioelectrical phenomena, as it is related to the important cellular events involved in the host defense system such as antigen processing, chemotaxis, and secretion. These techniques may allow the measurement of subtle changes in the host defense system caused by changes in macrophage activity in response to radiation. In addition, collaborative studies have resulted in indirect measurement of membrane potential in both neutrophils and macrophages, using membrane potential-sensitive fluorescent dyes, a relatively new and important tool for monitoring membrane potential in small cells.

BEHAVIORAL SCIENCES

Basic research efforts within the Behavioral Sciences Department are directed toward developing sensitive experimental models to study the effects of ionizing radiation on behavior and the identification of underlying biological mechanisms involved in producing these effects. Refinement of existing paradigms for studying the effects of radiation on behavior will allow more precise determination of thresholds and finer quantification of behavioral effects. Experimental models will be applied to the study of radiation-induced performance decrements and to problems associated with testing efficacy and potential toxicity of purported radioprotectants.

Since it is desirable to be able to predict human responses from the results of behavioral experiments on nonhuman subjects, experimental studies will be conducted to investigate cross-species comparisons using behavioral paradigms that have proven useful in the fields of behavioral toxicology and psychopharmacology. The goal will be the development of experimental models involving animal subjects, to produce results more directly applicable to man than is currently possible.

Advanced neurobehavioral techniques will be used to examine the effects of radiation on specific areas of the brain, with the goal of better understanding the mechanisms by which radiation exerts its effects on the behaving organism. A wide variety of multidisciplinary techniques are presently available or under development to study these mechanisms, requiring expertise in neurochemistry, neuropharmacology, neurophysiology, and neuroanatomy. This will allow a systematic study to determine which areas of the brain are sensitive to radiation and to detemrine which neurotransmitter systems are actively involved in specific behavioral abnormalities.

A method is currently under development that will allow the analysis of a number of neurotransmitters, their metabolites, and other relevant substances from a single tissue smaple. Using high-performance liquid chromatography (HPLC) with its high sensitivity, measurements will be possible in very small tissue samples representing tissue punches of individual nuclei in the brain.

To gain information on neurotransmitter dynamics in the behaving animal, push-pull cannulae wil be implanted in various areas of the brain, and the release of neurotransmitters will be measured in the perfusate by HPLC. In this way, specific neurochemical changes and pharmacological manipulation of precise areas of the brain may be detected.

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