III. Overview: What is Radiobiology?

The purpose of this web-based textbook is to provide resources for education, and also to encourage students at all levels to approach Radiation Biology and Radiation Biology Methods with enthusiasm to facilitate a better understanding of the discipline.

There are several available radiobiology textbooks, and web-based historical and overview educational tools. Accordingly, it is the purpose of this virtual textbook to highlight methods and methodologies used for data collection in each of the topics described in the chapter that follow. These methods chapters are designed to foster entry and help new scientists into the field of Radiobiology, helping them adapt to the use of new cutting-edge tools to further research. The Center for Medical Countermeasures Against Radiation Consortium (CMCR-C) topics of focused research (See Chapter XXXVI of this textbook) include: "Improved Radiation Dosimetry" and "Drug Discovery" including design of technologies for rapid measurement of radiation doses, and the development of new radiation countermeasures.

History:

Since the discovery of ionizing irradiation in 1895, the discipline of Radiobiology has evolved from both the clinical observations and advances in basic science. Radiobiology is the study of molecular, biochemical, cellular, tissue, organ, and total body responses to ionizing irradiation. Observations on clinical outcomes with those physicians using radium sources, and ortho-voltage x-rays during the turn of the 20th Century, brought into light the hazards of over-exposure to

ionizing irradiation (1). These observations alerted scientists to the concepts of radiation dose, dose rate, and volume of tissue exposed. The principles, which extended from clinical and veterinary medicine were published in the 1st decade of the 20th Century. X-ray exposure (both diagnostic and therapeutic) of different animal species including, experimental animals, pets, and livestock, brought to light the concept of species differences and age related differences in the ionizing irradiation response. Radiobiology evolved from basic principles, which were established during the years leading up to the Atomic Bomb explosions in 1945, and continued to the end of the Cold War in 1989 (1). The classic radiobiology scientific publications include those in radiation chemistry, cell biology, and biochemistry. The concept of the clonogenic radiation survival curve, induction of DNA strand breaks by ionizing irradiation, and the phenomenon of the various organ system "syndromes" were characterized during the 1940s to 1950s. Organ system failures were shown to be dependent on radiation dose, dose rate, and volume of tissue treated. The basic principles of Radiobiology have endured to the modern era.

The current definition of Radiobiology now encompasses nearly all the basic and clinical science disciplines associated with acute inflammation, organ system failures, infection, and most prominently, the mechanism(s) of radiation late effects. Radiation late effects are similar to clinical late effects that follow other forms of tissue injury, but have different mechanisms of etiology and are specific to each organ. These include: radiation fibrosis, stem cell depletion, physiologic failure, and cellular senescence. At the molecular level, Modern Radiobiology encompasses nearly all the defined principals of molecular, biochemical, cellular pathways and networks described in Basic Molecular Biology, Biochemistry, and Cellular Physiology.

The subject of Radiobiology must first reference the pioneering experiments of Theodore Puck, Mortimer Elkind, and with respect to the Radiation Countermeasures CMCR Consortium Program that of E. Donnall Thomas regarding bone marrow transplantation (1). Total body irradiation to doses, which induce failure of the hematopoietic (blood forming) system, and can be rescued by intravenous injection of single cell suspensions of bone marrow from a donor is termed the *Hematopoietic Syndrome*, as it is referenced in books of modern bone marrow transplantation. An entire sub-field of Clinical Hematology is now devoted to bone marrow transplantation. The subfield evolved from Radiobiology of marrow failure. Experiments done initially in rodents, dogs, then in non-human primates, and humans, led to the observation that there was a threshold dose of total body radiation dose below which bone marrow transplantation could save the life of an individual (1). Above this threshold dose (4-4.5 Gy in humans), another system failure, prevents rescue by marrow transplant namely the gastrointestinal or GI Syndrome. Therefore, the gastrointestinal system failure or "*GI syndrome*" as the cause of death following higher total body irradiation doses is not rescued by bone marrow transplantation.

Radiation Damage Categories:

The time courses leading to death from total body irradiation has been defined in animal model systems, and confirmed in documentation of the survivors of nuclear accidents. These data led to the design of broadly defined categories of organ system failure. The *hematopoietic syndrome* was defined as that which could be rescued by bone marrow transplantation. Sparing one area of bone marrow by concrete or lead shielding led to discovery of the hematopoietic stem cell (HSC), which could migrate through the blood from the shielded area of marrow and repopulate

the rest of the irradiated marrow volumes. The *GI syndrome*, was defined as that which was associated with incomplete restoration of intestinal villi in the ileum and jejunum, and which could not be rescued or healed by marrow transplant. Efforts to prevent death from the GI Syndrome focalized on treating infection from breakdown of the gastrointestinal lumen barrier, and entry of intestinal bacteria into the circulation to produce fatal septicemia. The *GI Syndrome* was not always rescued by antimicrobial drugs or by bone marrow transplantation leading to the concept of intestinal stem cell damage. Even higher total body irradiation doses above 10 Gy were associated with brain swelling, and death from seizures. There was a rapid systemic outpouring of inflammatory cytokines, and this event was termed the "*CNS or Central Nervous System Syndrome*".

From clinical observations with irradiated animals and humans suffering from radiation accidents in nuclear power plant facilites or nuclear weapon explosions, the data indicated that irradiation late effects compiled a separate category and were organ or organ system specific. The most prominent irradiation late effect was found to be organ failure and frequently involved histopathologic evidence of microscopic fibrosis. In attempts to understand the mechanism of fibrosis, elegant experiments with transgenic and knockout mouse strains were used and defined the importance of "pro-fibrotic" cytokines, principally transforming growth factor – Beta (TGF-β). Studying, late radiation effects has become prominent in current clinical investigations. Because of the importance of late effects, several clinical disciplines have joined forces to study this area. Chronic low dose rate radiation exposure from inhalation or ingestion of long-lived isotopes with a long half-line (defined as the time over which one half of the energy from productions of photons, protons, and alpha particles (Helium nuclei), neutrons has dissipated) is

one area of each research focus (1). Another area involves sporadic high dose rate irradiation events such as that found in space travel from galactic cosmic irradiation or solar proton events. Many ionizing radiation late effect observed in humans or animals models, have led to a new sub-category of targets for therapeutic intervention. These include study of the formation of cataracts (opacity in lens of the eye), renal failure, liver failure, neurodegenerative diseases, cellular senescence, and overall accelerated aging.

The most prominent late effect of ionizing irradiation was found to be cancer in both the lymphohematopoietic system (leukemogenesis) and the formation of solid tumors (carcinogenesis).

Modern Radiobiology, as a discipline is taught to radiation oncology and diagnostic radiology residents, and in basic science programs throughout the world. Radiobiology includes several categories of research.

Subcategories of Radiobiology:

The field of *Radiation Chemistry*, deals with the initial hydrolysis of water by ionizing irradiation to produce a reactive oxygen species and other highly unstable free radicals that interact with the nucleus of cells and induce DNA double strand breaks (1). These events occur within fractions of a section of ionizing radiation exposure and the number of DNA strand breaks is dependent upon radiation dose, defined in physical terms as ergs of energy per milligram of tissue or Joules per kilogram, all of which are measurable in fractions of a second. Radiation

Chemistry terms overlap with basic radiation physics terms. The category of ionizing radiation, which is low-LET or low linear energy transfer (LET) includes Gamma rays (photons measured as arising from decay or transmutation of radioisotopes), and x-rays (photons measured from physical generation by a beam of electrons hitting a tungsten or other metal target). X-rays produce energy dependent physical effects in tissues categorized by: photo-electric effect, Compton scattering, and pair production.

The field of *radiation physics* is critical to any effort to teach radiobiology, as it involves precision in measurement of x-rays, particle beams, as well as isotopes by detectors. This mandate led to the development of highly sensitive radiation detectors. Ionizing irradiation creates ionization of air, measurable by Geiger counters, but also using physical film dosimeters and thermoluminescent dosimeters (TLD), all of which are currently in use in Clinical Radiology and Radiation Oncology departments, as well as in Radiation Safety Offices throughout the world. Physical Dosimetry has become very accurate and safe with the capacity to reliably quantitate thousandths of a Gray (100 rads) of irradiation. Definitions of irradiation units have also evolved and will be discussed in this web-based book in the Chapters on Radiation Physics and Radiation Safety.

Modern Radiation Biology includes an emphasis on the Oxygen effect, and the Oxygen enhancement rates (OER) (1). This means that less Oxygen in the irradiated tissues produces less free radicals to damage DNA in that tissue <u>in</u> <u>vivo</u> or cells in tissue culture. Also, important is the relative biologic effect (RBE). All equivalent physical measured radiation quantities are not the same with respect to a specific effect in a biologic system: whether in a single cell,

tissue, or organ, and the effects also differ between species. Determining whether a given physical measure of radiation produces the same or a different specific biological outcome in an animal species or cell population led to the sub-category of radiation biology involving an understanding of mechanism by which cells, tissues, organs, organ systems, and communicate irradiation injury. For example, proton or neutron particle beams may generate physical measurements identical to photons (for example: 1 Gy), but are different from that same (1 Gy) measured as energy deposition caused by x-rays or gamma rays. Since the same physical dose is measured by a physical dosimeter from high LET particles such as (protons) compared to low LET photons (x-rays), it was discovered that high LET irradiation induced more direct nuclear DNA double strand breaks causing more damage and more biologic effects than that same physical dose induced by x-rays. The RBE of protons is considered to be higher than that of x-rays. Recent studies continue to show that higher RBE levels are even higher when the measured irradiation is generated by an accelerated atom (such as Iron 59 nuclei found as a component of galactic cosmic irradiation caused by the explosion of Supernova in the vacuum of space) or to Helium atom nuclei (also termed an alpha particles) compared to the mass-less or low mass (low LET) x-ray photons or gamma rays.

Methods in Areas of Radiobiological Research:

Several chapters in this web-based textbook have been designed to address each of the areas of Classical and Modern Radiobiology, while providing the reader new information on how each of these disciplines utilizes current (sometimes general basic science) methodologies. Methods section in each chapter will include instructions on how to perform these measurements, generate

reproducible data, and analyze data. For example, the sections on DNA double strand breaks and repair include: methodology for how such DNA strand breaks can be observed, quantitated, and all of the assays for the complex steps involved in repair. Other sections in this web-based book are designed to provide the reader with an understanding of the overlap in the stages and measured events during a response to ionizing irradiation. For example, initial radiation induction of DNA double strand breaks is followed by a cascade of molecular signals that involve communication from the cell nucleus to the cytoplasm including mitochondria endoplasmic reticulum and cell membrane.

A large section is devoted to mitochondrial responses to irradiation and emphasizes the importance of the several known cellular death pathways in the radiation response (apoptosis, necroptosis, ferroptosis, and necrosis. Each pathway has gained prominence in recent decades. Because of their involvement in cellular, tissue, and organ death from mechanisms other than ionizing irradiation, each death pathway is considered separately. The mechanisms of cell death, each of which is quantitated by different assays will also be addressed in a separate section of this book. We include a description of the techniques by which to carry out assays for each of these pathways including: apoptosis, necroptosis, necrosis, ferroptosis, and others.

Communication between irradiated cells, which can lead to deleterious acute and late effects will also be discussed. In a separate chapter of this book, both cell contact and cytokine (humoral factor release) will be shown to be involved in these processes. Organ specific and tissue volume specific responses to irradiation now also involves an understanding of cellular and humoral immunity.

The diversity of cell phenotypes within a tissue or organ necessitates an understanding of the irradiation response at the tissue level and involves study of each of several cell pheontypes: endothelial cells, stromal cells, stem cells, and differentiated cells. How cells communicate the damage signals locally within that organ, and how they mediate signaling systemically through release of humoral cytokines into the circulation will be addressed.

Both acute and chronic radiation responses involve cell death and growth or differentiation modifications (phenotypic differentiation, senescence, quiescence) of stem cells, sometimes specific to each organ or tissue. Stem cell biology has evolved as a complicated discipline and involves the understanding of modern techniques used in the study of embryonic stem cells, inducible human pluripotential stem cells, as well as antibody labeled cell flow analysis and cell sorting of stem cell populations taken out of the bone marrow or solid organs. Any discussion of late irradiation effects must now include an understanding of cellular senescence. The phenomenon of senescence, a process by which cells are unable to divide, but sit in tissues and release cytokines that can cause tissue damage and organ failure (including replacement of functioning tissue with scar tissue or radiation fibrosis) is very important in radiation biology. Accelerated senescence, induced by irradiation, leads to accelerated aging. Aging is another discipline, which must be included in the study of Modern Radiobiology. Assays for cellular senescence include description of the methodology for each technique, and are included in another section of the textbook.

An understanding of Radiobiology as applied to several clinical disciplines in Modern Medicine emphasizes many relevant safety concerns. As applied to Radiation Oncology, Diagnostic Radiology, Nuclear Medicine, and Cardiology (Fluoroscopy), the use of x-rays allows diagnostic and treatment procedures, as well as minimally invasive surgery, coronary and systemic vascular angioplasty, and microsurgery, but safety and dose monitoring is critical both for patient and care provider.

Radiation Countermeasures and Biodosimetry:

The clinical discipline of Radiation Oncology has led to the concept of the therapeutic ratio (tumor cell kill/normal cellular and tissue damage). This area led to the development of tumor radiosensitizers and normal tissue radioprotectors. Gene therapy approaches, as well as small molecule clinical pharmacology, have led to the development and clinical utilization of a variety of agents by which are used to improve the therapeutic ratio (more cancer cell death/less normal tissue damage) during cancer treatment using ionizing irradiation. These strategies have defined the whole field of tumor biology. This textbook will not address the use of ionizing irradiation in diagnoses or treatment of cancer, but will address the development of radiation mitigator drugs, principally small molecule radiation mitigators for radiation counter-terrorism; however, several of these drugs did arise from prior clinical applications of the agents in the treatment of cancer patients.

A major section of this web-based textbook will be devoted to new techniques for Radiation Biodosimetry (understanding the biological effects of irradiation and quantitating these effects in

animals and in humans), linked to the development and delivery of radiation countermeasures. A significant section will be devoted to the definition and understanding of radiation countermeasures, but also described as categories of such pharmaceuticals or biological response modifiers. The research discovery, development of such agents, their application, and quantification of effects will be addressed.

Readers of this web-based textbook are encouraged to communicate with the authors of each section to get more information. (Email addresses are attached to each author in Table of Contents.). The references in each chapter will be few, but will be designed to target review articles and major concept articles. Such review articles can then lead to primary references and allow the reader to "dig deeper" into each specific area.

References:

1. Hall, E.J., Giaccia, A.J. Radiobiology for the Radiologist, 6th Edition, Walters Kluwer, Lippincott, Williams, & Wilkins, 2006.