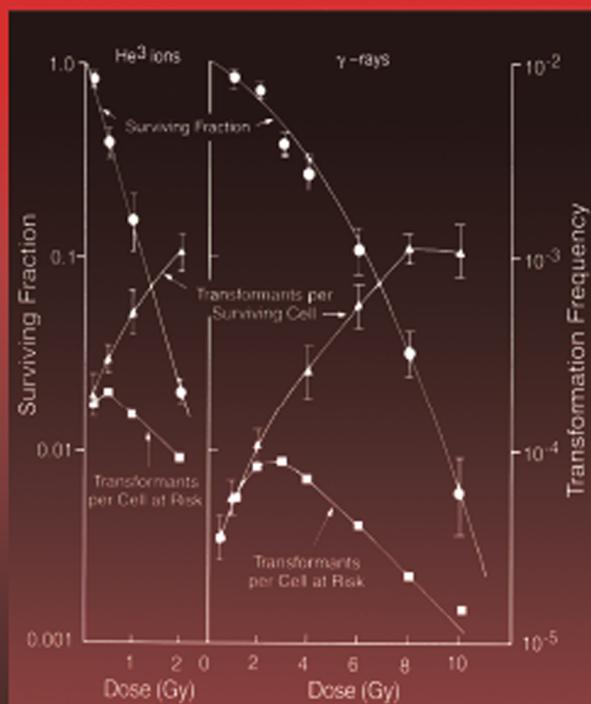
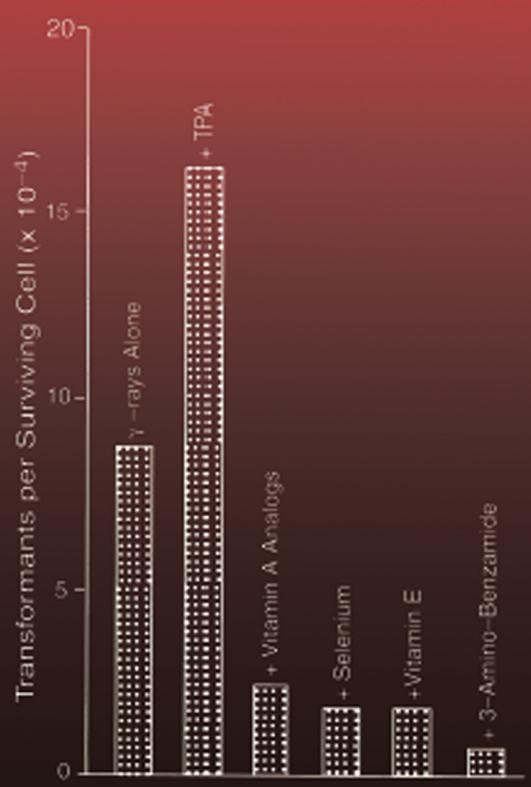


Handbook of **RADIOBIOLOGY**

Second Edition



Kedar N. Prasad

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PREFACE

The purpose of this handbook is to provide the most recent information on the effects of ionizing radiation on mammalian cells, with emphasis on human tissues. The dose-effect relationship has been emphasized in a quantitative manner. The book contains recent data on the late effects of low levels of radiation on humans. This handbook also contains some of the late consequences of radiation therapy which are detected among survivors of cancer patients. Recent studies on the interaction of radiation with hyperthermia, electronaffinic compounds, and other new radiomodifying agents have been analyzed and discussed. In addition, the effects of radiation on mutation, chromosomal damage, fetuses and cancer incidence have been critically reviewed. Recent advances in identification and, subsequently, availability of several proto-oncogenes and their products have provided new tools to investigate the mechanisms of action of ionizing radiation on mammalian cells. This book has summarized the effect of radiation on the expression of several proto-oncogenes and oncogenes. Radiation-induced carcinogenesis has been discussed in mechanistic terms, and a separate chapter describing current strategy for cancer prevention has been included. The revised concept and value of maximum permissible dose has been added. This would serve as a reference book for radiobiologists, residents in diagnostics, nuclear medicine and radiation oncology, and graduate students in radiation biology.

THE AUTHOR

Kedar N. Prasad, Ph.D., is Professor of Radiology and Director of the Center for Vitamins and Cancer Research, at the School of Medicine, University of Colorado Health Sciences Center, Denver. Dr. Prasad has done pioneering work on the modification of the effect of ionizing radiation and hyperthermia by physiological substances such as adenosine 3',5'-cyclic monophosphate (cAMP), butyric acid, vitamin C and vitamin E on some tumor cells in culture. In addition, he has published original work on the control mechanisms of differentiation and malignancy, using neuroblastoma cells in culture as an experimental model. He has made a highly significant contribution on the role of nutrition in cancer prevention and treatment. His current research involves immortalization of parotid acinar cells and nerve cells for transplantation studies. Dr. Prasad has published over 200 full publications, several reviews, and books in radiation biology and cell biology. He has edited six books on nutrition and cancer.

Dr. Prasad is a frequent speaker at a major national and international conference on cancer. He is a member of several national and international scientific organizations. He served as President of the International Association of Vitamins and Nutritional Oncology, and is currently serving as President of the International Society For Nutrition and Cancer.

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Chapter 1

DEVELOPMENT OF RADIOBIOLOGY: A REVIEW

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I. INTRODUCTION

The development of radiation biology began immediately after the discovery of the X-ray by Roentgen in 1895. Becquerel and Curie observed that certain substances (compounds of uranium, radium, and polonium) were naturally radioactive. Since then, the development of radiation biology has been linked with the advancement of nuclear physics and basic cell biology on the one hand and with the growing awareness of the hazards and usefulness of ionizing radiation on the other. Some of the major discoveries in nuclear physics^{16,17,24} and biology^{5,8,20,23,28-31} that have influenced the growth of radiation biology are briefly described.

II. MAJOR DISCOVERIES IN NUCLEAR PHYSICS

Soon after the discovery of the X-ray and naturally occurring radioactive substances, Thompson defined the physical properties of electrons and protons.¹⁷ In 1911, Rutherford, at the University of Cambridge, discovered alpha-particles, and in 1932 Chadwick made the

discovery of neutrons.¹⁷ The availability of neutrons made possible the production of several radioisotopes of biological and medical interest. Also, the relative biologic effectiveness of neutrons with respect to the X-ray was investigated. In 1932, the invention of particle accelerators (the cyclotron) by Lawrence at the University of California, Berkeley, was of great significance.¹⁷ Since then, the cyclotron has been used as a means of production of several radioisotopes of biological and medical interest. Also, the relative biological effectiveness of neutrons with respect to the X-ray was investigated. On December 2, 1942, Fermi and associates at the University of Chicago accomplished a chain reaction from the fission of uranium atoms in a pile of graphite blocks. This remarkable discovery became the basis for manufacturing the atom bomb and the nuclear reactor. Today, most of the radioisotopes of biological and medical interest are produced in the nuclear reactor. In addition to this, the nuclear reactor serves as a source of neutrons of different energies that are being utilized for the study of radiation injuries.

Recent advances in accelerator technology make possible the attainment of very high-intensity proton beams. Such proton beams are adequate for providing pure, high-intensity beams of negative pions (π^-). The accelerator, which produces π , is referred to as a "meson factory"²⁴ and is now in use at the Los Alamos Scientific Laboratory, New Mexico. Theoretically, it appears that such a beam could deposit, at essentially any depth in animals and humans, more energy than could be deposited by other particles such as protons, neutrons, and alpha particles. This is due to the fact that when a negative pion is captured by an oxygen nucleus, the mass of the pion is converted into energy with a consequent violent disruption of the oxygen nucleus. From the nucleus emerge neutrons, protons, alpha particles, Li, Be, and C ions; however, the dominant mode involves alpha particles, which have short range. Negative pions can be used in radiobiological studies.

The availability of a variety of radioisotopes has served both as a source of radiation for evaluating the biological hazards of ionizing radiation and as a tracer for the study of the function of various organs and cells.²² It has also helped in providing a better knowledge of the mechanisms of radiation injuries. Radioactive-labeled antibodies are being used in the treatment of human cancers as well as in animal cancer.

Our dosimetry has become accurate;²⁶ therefore, we can establish an accurate dose-effect relationship.

III. MAJOR DISCOVERIES IN BIOLOGY

Several major advances in cellular and molecular biology have markedly influenced the development of radiation biology. For example, the establishment of the mammalian cell line *in vitro* and the identification of various phases in the life cycle of a cell have increased our understanding of cellular radiosensitivity.⁸ The study of ultrastructures of a cell by an electronic microscope has been very useful in investigating radiation injuries on a subcellular level. Although radiation-induced changes in the ultrastructures of a cell appear nonspecific, these cellular alterations, in combination with biochemical ones, have increased our understanding of radiation injuries. Radiobiologists have not yet taken advantage of the scanning electron microscope, which shows the surface structure of entire cells in great detail.

In 1953, the discovery of the double-helix model of DNA structure had a major impact³⁰ on the development of radiation biology. The structure of DNA and the mechanism of its replication have contributed to our understanding of the mechanisms of radiation damage and repair. The elucidation of protein biosynthesis³⁰ has also increased our knowledge of the mechanisms of radiation damage on the molecular level. The effects of irradiation on biosynthesis and kinetics of nucleic acid and protein synthesis have continued to be studied.

It is now established^{19,20} that mitochondria contain DNA that is capable of coding at least certain mitochondrial proteins. This is substantiated by the fact that mitochondria synthesize RNA and protein *in vitro*. The radiosensitivity of mitochondria has been studied primarily on the basis of morphologic changes, oxidative phosphorylation, and ion transportation, but the effects of irradiation on the biosynthesis of mitochondrial nucleic acid and protein have not been investigated.

Many studies have been performed on the effect of hormones in the regulation of cellular RNA and protein synthesis.^{28,29} Several hormones increase enzyme synthesis, which is related to an increased nuclear RNA synthesis. The radiosensitivity of newly formed RNA and protein has not been adequately investigated. Because hormones play an important role in the regulation of the metabolic functions of the cell, such a study would increase our understanding of cellular radiation damage and repair. Studies have established that cyclic nucleotides, adenosine 3',5'-cyclic monophosphate (cAMP), and guanosine 3',5'-monophosphate (cGMP) are important for several cellular functions. The importance of cyclic nucleotides in the modification of radiation response has not been adequately studied. These cyclic nucleotides affect the growth, morphology, and differentiation of mammalian cells in culture. The role played by cAMP and cGMP in the radiosensitivity of cells would be important to investigate in order to understand more about the mechanism of radiation damage. In addition, several new growth factors and some new protein kinases have been identified and isolated. The significance of these factors and kinases in modifying the radiation injury has just begun to be studied. The viral oncogenes probes have helped to identify several cellular oncogenes such as *c-ras* and *c-myc*. The techniques of molecular biology have been responsible for identifying new cellular protooncogenes. The role of these genes in radiosensitivity has not been studied adequately. The proteolytic activity of RNA under certain experimental conditions was another landmark discovery in biology.

The technique of somatic cell hybridization²³ of two different cell types may prove a very useful tool in obtaining some new insights regarding the radiosensitivity of mammalian cells. Hybrids are produced by fusing two cell types in the presence of an inactivated Sendai virus.

Radiation has contributed directly to the understanding of several aspects of cell biology that would have been difficult to understand by other means. Because radiation is efficient in killing only certain types of cells, the importance of such cells can be more easily evaluated by radiation rather than by other agents such as chemicals, which kill cells nonspecifically. On the basis of this principle, a map of organogenesis has been prepared by irradiating the embryo at different stages of development. Radiation also induces a high incidence of mutation; therefore, it has contributed considerably to our knowledge of mutagenic processes. For example, today we know that mice are much more sensitive to radiation-induced mutation than *Drosophila*. In addition, the repair of premutational changes occurs in mice.

The use of ³H-thymidine has helped in identifying various phases of the cell cycle and in estimating the period of each phase. The use of other radioactive-labeled compounds as a tracer has increased our understanding of the physiological, biochemical, and metabolical functions of various organs. The use of a lethal dose of X-radiation has helped to remove the presence of dividing cells from the differentiated neuroblastoma cell population in order to make them useful in the studies of neural transplantation.

IV. AWARENESS OF HAZARDS AND USEFULNESS OF RADIATION

Isolated cases of radiation lesions were observed soon after discovery of the X-ray. Both Becquerel and Curie suffered from acute *radiation dermatitis*, or so-called *radium burn*. These lesions appeared on areas continuously exposed to radiation. The first case of radiation

sickness was described 6 years after the discovery of the X-ray. The incidence of radiation injury increased considerably following Rutherford's discovery of artificial nuclear fission and Frederick and Irene Joliet Curie's discovery of how to obtain radioactivity artificially.

The earliest known case of radiation-induced cancer was reported in 1902. Curie herself died of aplastic anemia, which was probably due to prolonged exposure to radiation. Nine deaths due to bone cancer were recorded between 1922 and 1924 among watch industry workers who painted dials with radium. Constant licking of the radium brush during painting procedures led to the accumulation of large amounts of radium in the bones over a long period of time. Irradiation of bones induced bone cancer. Jacob Furth induced leukemia for the first time, in mice, by a single whole-body exposure of 400 R or by a closely spaced fractionated dose of 800 R. In the early days, a high incidence of skin cancer and leukemia was observed among radiologists who were exposed to chronic doses of X-rays during the course of their work. In recent years, radiation-induced carcinogenesis has been extensively studied.^{6,12} Furthermore, antioxidant vitamins appear to modify the effect of radiation and reduce the risk of radiation-induced cancer. Today, growing awareness of the hazards of radiation and improved safety devices for radiation sources have reduced the risk of radiation-induced neoplastic disease among radiation workers. Schwarz²⁶ published an excellent review, *Radiation Hazards to the Human Fetus in Present-Day Society*, in which he discussed the hazards of diagnostic X-rays in pregnant women. He concluded that the diagnostic X-ray is very harmful for the fetus, and therefore pregnant women should not receive diagnostic X-rays unless it is absolutely essential for their health. The BEIR (Biological Effect of Ionizing Radiation) Committee report² also extensively discussed the effects of low levels of radiation on humans. This is the most authoritative source of estimation of low-dose radiation injuries in humans.

The discovery that radiation-induced gene mutation in *Drosophila* further dramatized the hazards of radiation. When animals were used to study radiation-induced mutation, some new concepts emerged.

The great tragedy caused by the atomic bombardment of Hiroshima and Nagasaki aroused serious concern among physicists, biologists, and the public. This led to the rapid expansion of radiation biology, the primary purpose of which was to evaluate the possible hazards of radiation and to understand the mechanisms of radiation injury. Human data on the biologic effects of single whole-body radiation exposure came primarily from the people of Hiroshima and Nagasaki who were exposed at the time of atomic explosion.

It should be emphasized, however, that radiation has been useful in biology and medicine. Henri Coutard was the first to develop the "fractionation dose technique," which involves the administration of daily fractional doses of X-rays. This allows the delivery of large radiation doses in "the most effective period of time." The purpose of such a radiation regime was to destroy the tumor while inflicting minimal permanent damage to the skin and other normal tissue. This type of therapy would be more effective if the radiation therapist had some knowledge of cell kinetics and/or if nontoxic radioprotectors — which can selectively protect normal tissue, or radiosensitizers (which can selectively sensitize the effect of radiation, preferentially, on tumor cells) — are found. Unfortunately, the estimation of cell kinetics in human tumors *in vivo* is extremely difficult. Radiation is being used extensively in the diagnosis of several diseases. Early diagnosis of many diseases has cured patients and has prolonged their lives.

V. AGRICULTURE AND FOOD PRESERVATION

Radiation induces mutation in both plants and animals. Although most mutations are deleterious, careful selection and breeding of beneficial mutants have led to the production of

mutant strains that produce a greater yield of crops than the wild type. Several studies have shown the possibility of using a massive dose of radiation for food preservation.

VI. SOME MAJOR DEVELOPMENTS IN RADIATION BIOLOGY

A. LAW OF BERGONIÉ AND TRIBONDÉAU

As early as 1906 the French scientists Bergonié and Tribondéau, working with rat testis, proposed a new hypothesis on the radiosensitivity of cells, which in broad terms is as follows: (1) less differentiated cells are more radiosensitive than highly differentiated ones, and (2) proliferating tissues are more radiosensitive than nonproliferating ones. The generality of this law is still true, with the exception of lymphocytes and oocytes, which are very radiosensitive in spite of the fact that they are highly differentiated and are not dividing.

B. TARGET THEORY

To explain the biologic effects of ionizing radiation, several ideas were introduced. Among these, the concept of target theory originally proposed by Dessaur in 1922 and later expanded by Lea¹⁸ proved useful in the study of radiation biology. This theory in its simplest terms predicts that inactivation of biological molecules increases exponentially as a function of dose. This theory assumes that the inactivation of the molecules is caused by a direct hit and, therefore, is also referred to as "direct action" or "direct effect."

C. INDIRECT EFFECT

The target theory was found inadequate to explain cellular radiation injuries. Dale, Evans, and Gray developed the concept of indirect effect or indirect action radiation,^{1,7,10,13,15,25} according to which biologic molecules are inactivated by free radicals, which are formed when radiation interacts with water.

D. RELATIONSHIP BETWEEN CHROMOSOME VOLUME AND RADIOSENSITIVITY

On the basis of target theory, Sparrow²⁷ proposed a new hypothesis to explain some of the discrepancies in the radiosensitivity of various species. According to his hypothesis, the radiosensitivity of a cell is directly proportional to its interphase chromosomal volume. This hypothesis is consistent with his observations on several plant species. He further speculated that if one expresses the dose as energy absorption per chromosome, an apparent difference in the radiation response of various animal species may largely disappear. The data obtained from several plant species are consistent with Sparrow's hypothesis; however, the validity of this hypothesis for mammalian species remains to be established.

E. OXYGEN EFFECT

Oxygenated tissues were more sensitive to irradiation than hypoxic ones.⁸ This finding has become a theoretical basis for hyperbaric therapy of those tumors that have hypoxic cells.

F. CONCEPT OF RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)

The concept of RBE evolved because of the availability of several types of radiation that produce different degrees of damage with the same dose. This is due to the fact that the linear energy transfer (LET) for each type of radiation is different. For the same total dose, the radiation of high LET (alpha particles, protons) produces greater damage than that of low LET radiation (X- and gamma ray). In addition, the oxygen effect, which is so marked with the radiation of low LET, is negligible with radiation of high LET.

G. MODIFICATION OF RADIATION DAMAGE

The discovery of several radioprotective³¹ and radiosensitizing agents^{1,10,25} has increased our knowledge of radiation injuries. Extensive work has been done on radiation injuries of the small intestine and bone marrow.³ Bond et al.³ have recommended an excellent therapeutic regime for accidentally exposed individuals. This involves a "functional replacement therapy," which requires transfusions of fresh platelets, whole blood, and antibiotics whenever needed. Spleen, spleen cells, and bone marrow transplantation protect animals after exposure.¹⁰ Cell-free spleen extract as a radiation therapeutic agent was first shown by Ellinger⁹ and was confirmed by Ford et al.¹¹ Several new modifying agents have been identified.^{4,14} Electronaffinic compounds are in clinical trials, but the results have been disappointing.

H. QUANTITATIVE RADIATION BIOLOGY

The development of quantitative radiation biology owes much to the discovery of the colony technique. This technique measures the reproductive integrity of irradiated cells and is commonly used in radiobiology. Recently, the technique⁸ of counting the number of colony-forming units (CFU) in the spleen of lethally irradiated mice was also developed.⁸ This method provided a very useful tool in the assaying of radiation injuries of the spleen and bone marrow *in vivo*. In addition to these biologic parameters, electron paramagnetic resonance (EPR) is being used to measure the free radicals produced in irradiated materials.

I. CELLULAR RADIOSENSITIVITY AND CELLULAR REPAIR

Success in identifying various phases of the cell cycle and in culturing synchronized mammalian cells *in vitro* has provided new information regarding the radiosensitivity of cells in relation to the cell cycle.⁸ On the criterion of cell death, the mitosis phase of the cell cycle is considered to be the most radiosensitive; however, on other criteria such as reduction of DNA synthesis or chromosomal damage, this may not be true. Like bacteria, mammalian cells repair radiation damage.^{8,21} Mammalian cells *in vitro* repair sublethal and potentially lethal damage.

VII. SUMMARY AND COMMENTS

In our nuclear era, radiation biology will continue to grow as an important field in modern biology. The extensive use of atomic energy in various branches of the national economy, technology, science, biology, and medicine has made the study of radiation injury and radiation protection an important subject. It is for this reason that biologists, physicians, physicists, and chemists are working together in the area of radiation research to obtain a better understanding of radiation injuries and their modifications. Close collaboration between radiation biologists and radiation therapists has become necessary for the most effective treatment of neoplastic diseases, but one has to constantly remember that while radiation treats cancer, at the same time it has the potential to induce cancer. Therefore, radiation should be used only when necessary, and all measures must be taken to minimize the exposure of normal tissues. To increase the efficiency of radiation therapy, investigators are studying three major areas: (1) radioprotective agents, (2) radiosensitizing agents, and (3) the effect of high LET radiation. With the growing use of nuclear energy in industry, technology, and the sciences, radiation biology will continue to grow. The author believes that the current emphasis on the modification of radiation injury of tumor and normal cells will eventually increase the efficacy of radiation therapy. Based on our present knowledge of radiation effects, the maximum permissible dose (MPD) is recommended as an "acceptable risk." Therefore, the benefit from radiation must be overwhelming before an individual or the public is exposed to the MPD. Because there is no radiation dose known as "safe," continuous effort must be made to

minimize the exposure level as much as possible; the extent to which these efforts are successful may very well affect the whole future of nuclear energy.

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Chapter 2
BASIC CELL BIOLOGY

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I. INTRODUCTION

To understand the radiation injury of cells, one must be thoroughly aware of their structures, kinetics, and functions. Therefore, a brief description of basic cell biology in its simplest form is presented in this chapter.

II. ULTRASTRUCTURE OF A CELL

The electron microscope has provided a powerful tool in the study of subcellular structures of a cell. The most common cytoplasmic organelles, such as mitochondria, smooth and rough endoplasmic reticulum, ribosomes, polysomes, and Golgi apparatus, are seen (Figure 2.1). These structures may differ in quantity from one cell to another, but qualitatively they are common to all cell types. However, in certain highly specialized cells such as melanocytes, new subcellular structures (premelanosomes, melanosomes, and melanin granules) are present in addition to the usual cytoplasmic organelles.

A. MITOCHONDRIA

The structures and functions of mitochondria have been studied extensively.¹⁸ Each mitochondrion has a double membrane. The outer membrane is continuous and forms a sac, whereas the inner membrane has many infoldings — some of which have formed septa. These infoldings have been referred to as "cristae." The matrix in most mitochondria is continuous throughout the lumen.

Mitochondria contain the complete machinery for orderly oxidation of pyruvate and fatty acid via the Krebs cycle, including enzymes, coenzymes, and essential metals. During oxidation processes, adenosine triphosphate (ATP) is generated and is utilized in the metabolic function of cells. In addition, the mitochondria accumulate certain ions such as K⁺, Ca²⁺, Mg²⁺, and HPO₄²⁻, by active transport mechanisms; they thereby participate in the homeostatic regulation of ions in cells.

Mitochondria contain DNA, which, like bacteria, is circular in shape. The mitochondrial DNA is capable of coding the information for the biosynthesis of some mitochondrial proteins.^{22b} Indeed, it has been shown that mitochondria synthesize RNA and protein *in vitro*. These studies indicate that mitochondria occupy semiautonomous status within the cell. Further work is being done to elucidate the role of mitochondrial DNA in cellular metabolism.

B. RIBOSOMES AND POLYSOMES

Ribosomes (also referred to as ribonucleoprotein or microsomes) from widely different organisms are remarkably uniform in their general properties.¹⁷ These particles are composed

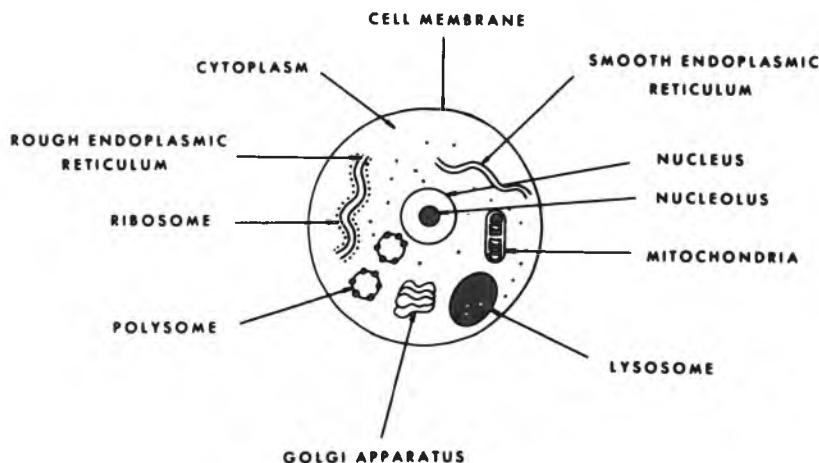


FIGURE 2.1. Diagrammatic representation of the ultrastructures of a cell. The following structures are seen: nucleus, nucleolus, nuclear membrane, chromatin materials, mitochondria, Golgi apparatus, rough endoplasmic reticulum, ribosomes, polysomes, and lysosomes.

of 40% protein and 60% RNA, and they constitute 80–90% of cellular RNA. Ribosomes are metabolically stable and occur in the cell either as a free form (70 S) or as subunits (50 S and 30 S). During the process of protein synthesis, more than one ribosome is present on the messenger RNA (mRNA) strand, and ribosome-mRNA complexes are referred to as poly-somes.

C. ENDOPLASMIC RETICULUM

The endoplasmic reticulum is an elongated membrane structure and occurs with or without the association of ribosomes; the former is called rough endoplasmic reticulum, whereas the latter is referred to as smooth endoplasmic reticulum. It has been suggested that these structures, at least in the rat liver, participate in the transportation of synthesized protein.

D. LYSOSOME

Lysosomes contain a number of hydrolytic enzymes, particularly acid phosphatase.⁸ They are found in a wide variety of tissue and participate in the removal of unwanted cellular materials. Rupture of a lysosome releases the hydrolytic enzymes, which may cause cell lethality.

E. GOLGI APPARATUS

In an electron micrograph, the Golgi apparatus exhibits a variable appearance. It consists of a collection of double membranes, large vacuoles, small vesicles, and granules. These structures participate in the secretory activity and increase in size during the elaboration of secretory substances by the thyroid. These organelles may also serve as a condensation center for materials being absorbed by the cells.

F. STRUCTURE OF A NUCLEUS

The nucleus consists of a nuclear membrane, nuclear sap, one or more nucleoli, and small granular elements called chromatins. The basic proteins of the nucleus appear homogeneously electron-dense when stained with osmic acid. The nuclear membrane is generally thicker than the plasma membrane surrounding the cytoplasm. The nuclear sap is usually more viscous than the cytoplasm. The nucleoli are round, dense, and well-defined bodies that are composed of RNA and associated proteins. The chromatin granules are composed of DNA and associated basic proteins. The nucleus contains the genetic material DNA and is essential for metabolic function of the cell. The nucleus is also necessary for cell division.

III. MITOSIS

The nucleus of a cell has a chromosome set that differs from one species to another. For example, a human has 46 chromosomes, whereas the mouse contains 40 and the golden pea only 14. Chemically, chromosomes contain not only DNA and histones, but also RNA and other proteins. It is well-known that DNA is the genetic material that is responsible for the heredity of characters. Therefore, any change in the structure of DNA of the germinal cell (spermatozoa or ova) would be manifested in the offspring. However, if DNA of the somatic cells such as skin, liver, and intestine has changed, such alterations would not be transferred to the offspring.

In mitosis, each chromosome duplicates itself. The duplicated strands separate as the nucleus divides, so that the daughter nuclei have the same set of chromosomes as their parent cell. Figure 2.2 shows a diagrammatic representation of the process of mitosis in a cell. During mitosis, a cell passes through four stages: prophase, metaphase, anaphase, and telophase.

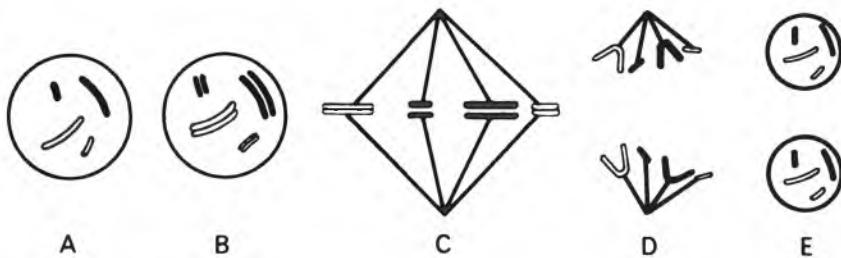


FIGURE 2.2. In mitosis, each chromosome duplicates itself. The duplicates separate as the nucleus divides, so the daughter nuclei are identical in chromosomal constitution. Prophase, A, B; metaphase, C; anaphase, D; and telophase, E. (From Sharp, L.W. *Fundamentals of Cytology*, McGraw-Hill, New York, 1943, 64. With permission.)

During prophase, each chromosome doubles itself, and the nuclear membrane and nucleus disappear. During metaphase, spindles form and chromosomes lie on the equatorial plate. During anaphase, chromosomes separate, and each half moves toward a pole. During telophase, the nucleus appears, and the cell divides into two daughter cells — each having an identical set of diploid chromosomes. The process of mitosis is so precise that any change in the chromosomes or DNA would definitely reflect in daughter cells after completion of cell division.

IV. MEIOSIS

This kind of nuclear division occurs only in the germinal cells (ovary and testis). In the testis during meiosis, each member of a paired chromosome duplicates, and the duplicated members come to lie side by side in a four-stranded configuration. The successive nuclear divisions result in the formation of four sperm, each with a haploid set of chromosomes (half of the parent cell). During meiosis, the first nuclear division is a mitotic one in which each daughter cell receives an identical set of diploid chromosomes. The second nuclear division is a reduction division in which each daughter cell contains only the haploid set of chromosomes. Diagrammatic representations of meiosis in the testis and ovary are shown in Figures 2.3 and 2.4. In the testis, spermatogonia divide by mitosis to form primary spermatocytes, which undergo reduction division to form spermatids. Spermatids have a haploid set of chromosomes. The spermatids undergo a maturation process to form spermatozoa. The entire process of the formation of spermatozoa is called spermatogenesis. The basic process of meiosis in the female is the same, except that each oocyte gives rise to only one functional egg, whereas each spermatocyte produces four functional spermatozoa. The process of forming the functional egg is called oogenesis.

V. CELL CYCLE

The life cycle of a cell is divided into four phases.¹² These include DNA synthetic phase (S), pre-DNA synthetic phase (G_1), post-DNA synthetic phase (G_2), and mitosis (M). A diagrammatic representation of the four phases of the cell cycle is shown in Figure 2.5.

Recently, another phase (G_0) has been identified in the cell population. This period is referred to as the “no-growth” period and signifies a time after mitosis, but before the onset of G_1 . The G_0 period is not part of the cell cycle, therefore it should not be included in the generation time, which represents the growth cycle only. The concept of G_0 is described in

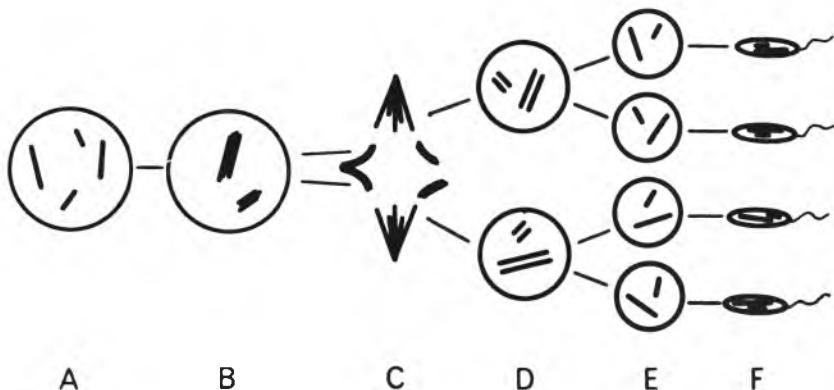


FIGURE 2.3. In the formation of sperm, duplicated members of each pair of chromosomes come to lie side by side in four-strand configurations (B). Two successive nuclear divisions result in the formation of four sperm (E), each with one member of each pair of chromosomes. The first division (C,D) is mitotic, whereas the second division is reduction (E). (From Sharp, L.W., *Introduction to Cytology*, McGraw-Hill, New York, 1934, 251. With permission.)

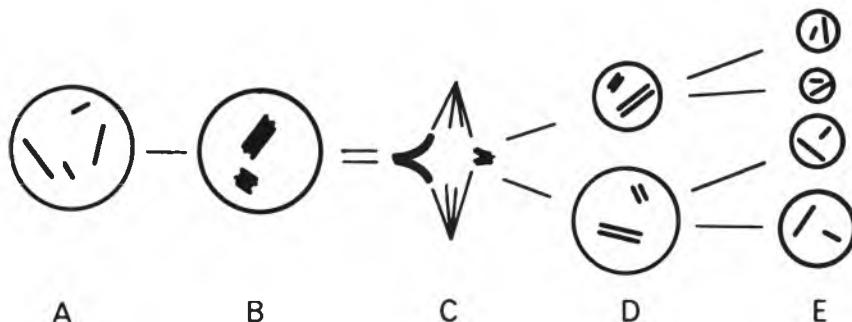


FIGURE 2.4. Meiosis in a female animal gives rise to only one functional egg from each primary oocyte and three polar bodies (E). Duplicated members of each chromosome lie side by side in four-strand configurations (B). The first division is mitotic (C, D), whereas the second division is reduction (E). (From Sharp, L.W., *Introduction to Cytology*, McGraw-Hill, New York, 1934, 251. With permission.)

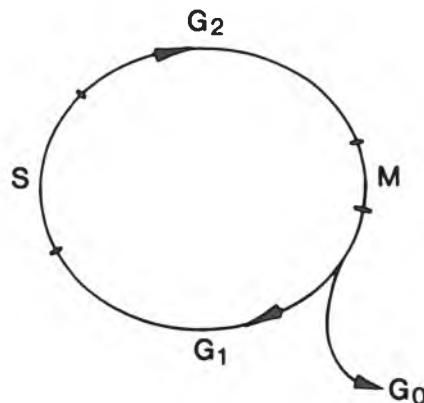


FIGURE 2.5. Diagrammatic representation of a cell cycle. The end of mitosis (M) marks the beginning of the cycle. A gap (G₁) occurs before the onset of DNA synthesis (S). DNA synthesis is followed by another gap (G₂) (post-DNA synthesis phase).

detail in a recent review.⁹ In brief, G_0 cells are those that are not dividing, but are capable of dividing or entering the cell cycle after a proper stimulus. A significant portion of tumor cells in a tumor mass is in the G_0 phase of the cell cycle. These cells are considered very resistant to X-rays and gamma rays.

There is a growing bulk of evidence that cell cycle time for a given cell type remains substantially constant. More recent works have shown that the G_1 phase is most sensitive to change. A large variation in the G_1 phase of transplanted tumor cells occurs, whereas the S, G_2 , and M phases are fairly constant. It has been shown¹⁶ that a hydrocortisone-induced increase in doubling time of HeLa Chessen cells is primarily due to an extension of the late G_1 phase of the cycle.

The doubling time refers to the average time taken for the cell number in a population to double. The generation time refers to the average time taken for cells to complete one growth cycle (G_1 -S- G_2 -M). Doubling time equals generation time if the following criteria are met in a given cell population: (1) growth is strictly exponential and devoid of fluctuation, (2) all cells are viable, and (3) all cells have the same generation time. The generation time is usually shorter than the doubling time.

The most common method of determining the generation time and the period of each phase of the cycle is the labeled mitosis method (Figure 2.6). Cells are pulse-labeled with ^{3}H -thymidine, and autoradiographs are prepared as a function of time after ^{3}H -thymidine injection. The labeled mitoses are plotted as a function of time after treatment with ^{3}H -thymidine. From this cyclic curve, generation time (T), G_2 , and S are estimated as indicated in Figure 2.6. For an exponential distribution of cells, the mitotic index (M) is calculated as follows: $M = (T/0.693) \times \text{mitotic index}$. After obtaining these values, one can determine $G_1 = T - (G_2 + S + M)$. Table 2.1 compares the generation time and the period of each phase of the cycle in various cell types.

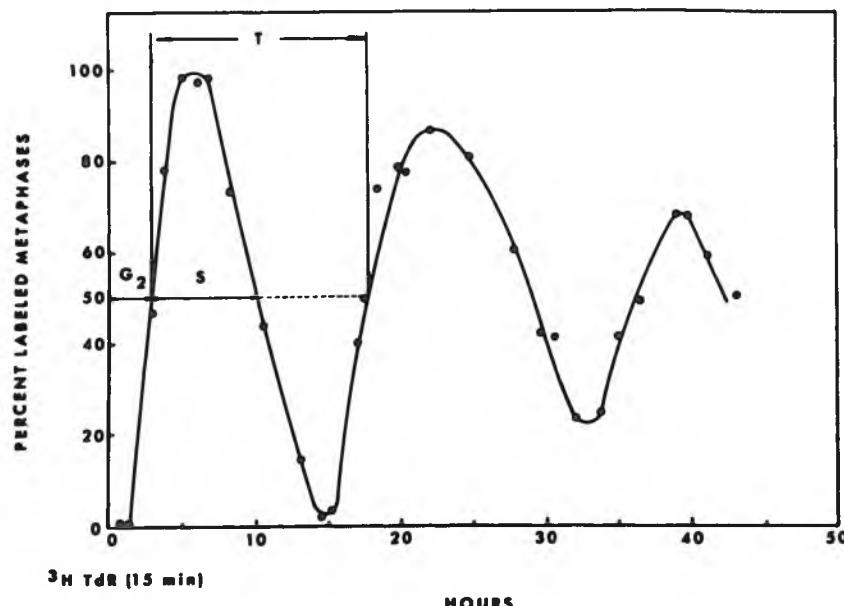


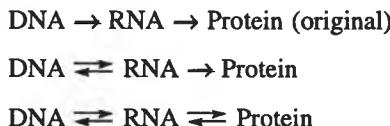
FIGURE 2.6. The percent of labeled metaphase as a function of time in a mouse L cell culture exposed to ^{3}H -thymidine for 15 min at time zero. T is the average generation time. (From Till, J.E., Whitmore, G., and Gulyas, S., *Biochem. Biophys. Acta*, 72, 277, 1963. With permission.)

TABLE 2.1
Cell Cycle Parameter

	Cell Type				
	G ₁	S	G ₂	M	T
Mouse cells	9.5	7	3	0.5	20
In culture	8.2	6.2	4.6	0.6	19.6
HeLa	3	7	1.5	0.5	12
Mouse hair follicle	3	7	1.5	0.5	12
Ehrlich ascites tumor	3	8.5	1.5	1	18

VI. NUCLEIC ACID AND PROTEIN SYNTHESIS

To understand the mechanisms of radiation damage, a basic knowledge of nucleic acid and protein synthesis is necessary.⁴¹ Therefore, the mechanisms of biosynthesis of DNA, RNA, and protein are presented here briefly. A diagrammatic representation of the molecular events for protein synthesis in the cell is given below.



A. DNA SYNTHESIS

DNA is a long chain of nucleotides and therefore is referred to as a polynucleotide. Each nucleotide is composed of three compounds linked together: (1) phosphoric acid; (2) a sugar, in the form of deoxyribose; and (3) a base (Figure 2.7). Bases are divided into two classes, purine and pyrimidine. In DNA, purines are adenine (A) and guanine (G), and pyrimidines are cytosine (C) and thymine (T). Thymine is very specific for DNA structure; therefore, ³H-thymidine has been used extensively in the study of DNA synthesis. In 1953, Watson and Crick proposed a double-helix structure for DNA in which two bases are joined together by hydrogen bonds.⁴³ A diagrammatic structure of DNA is shown in Figure 2.8. It should be noted that adenine pairs with thymine, whereas cytosine pairs with guanine. Figure 2.9 shows a schematic representation of DNA replication. During replication of each strand, the newly formed strand attaches with the proper base of the old one to form a double-stranded DNA. A new DNA strand was synthesized *in vitro* by using a specific enzyme polynucleotide pyrophosphorylase, precursor of DNA, and DNA from other sources. The newly formed DNA was identical to DNA that was added to the reaction mixture. This showed conclusively that DNA acts as a template for the synthesis of another strand of DNA.

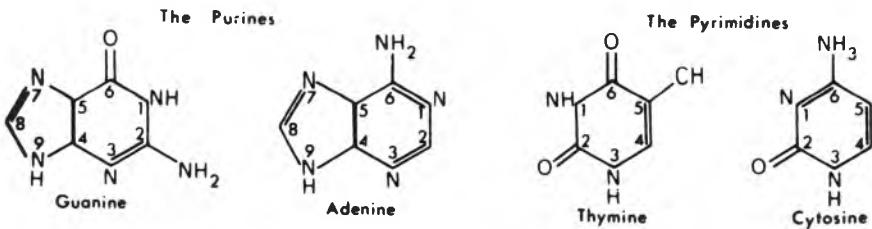
Several enzymes are required for DNA synthesis. The enzymes thymidine kinase and DNA polymerase have been studied in relation to radiation damage in some detail. DNA is degraded by a specific enzyme referred to as DNase (deoxyribonuclease).

The amount of DNA per nucleus within a given species is fairly constant; however, it varies markedly from one species to another.

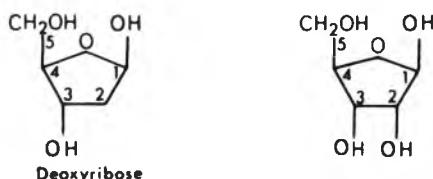
B. RNA AND PROTEIN SYNTHESIS

Like DNA, RNA is also a polynucleotide chain and consists of four bases, sugar, and phosphoric acid. RNA differs from DNA in the following respects: (1) it has sugar in the form of ribose rather than deoxyribose, and (2) it has pyrimidine base uracil in place of thymine.

THE BASES



THE SUGARS



A NUCLEOTIDE

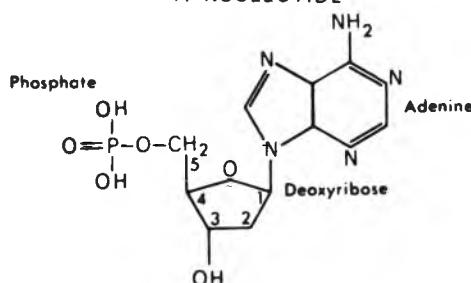


FIGURE 2.7. The chemical composition of nucleic acid. The bases are linked to a sugar and a phosphate to form a nucleotide. Nucleotides are linked together to form a nucleic acid or polynucleotide chain.

The enzyme RNA polymerase is required for RNA synthesis, and the enzyme RNase degrades RNA. There are several classes of mammalian RNA, three of which are most important: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). All these types of RNA participate in protein biosynthesis.

1. mRNA

It is now established that all information for protein synthesis is coded in DNA in the form of triplets (any combination of three bases). mRNA is synthesized on a DNA strand and can form a DNA/RNA hybrid *in vitro*. This species of RNA is called a *messenger* RNA because of its intermediary role in relaying the genetic code for protein synthesis to the functional site in the cytoplasm; mRNA brings the genetic code from DNA in the form of a triplet. For example, UUU dictates the incorporation of phenylalanine and CCC of proline and no other amino acid.

2. tRNA and rRNA

Transfer RNA picks up an activated amino acid in the cytoplasm. Each tRNA is specific for an amino acid, and tRNA carrying an amino acid attaches to a ribosome (30 S), which

moves along the mRNA strand and picks up a 50 S ribosome along the way; the tRNA-ribosome complex recognizes a particular code on the mRNA strand and transfers the amino acid to the growing polypeptide chain. tRNA and the ribosome come to lie in the cytoplasm and again repeat the sequence. A ribosome can exist in the cytoplasm as a free form (70 S) or in the form of subunits (50 S and 30 S).

3. Stability of mRNA

The concept of the stability of mRNA has changed markedly since it was originally proposed from studies in bacteria where the turnover of mRNA is very rapid, the half-life being 3 to 4 min. However, in mammalian cells, the half-life of mRNA is relatively long and often varies from one species to another. The half-life of mRNA in the mammalian liver is in the range 4–40 hr.⁷ In the amphibian liver (*Amphiuma tridactylum*), protein synthesis continues to occur at a normal rate for 96 hr after the production of new RNA is blocked by actinomycin D.²⁸ In some viruses RNA acts as a template for DNA synthesis via reverse transcriptase. The most recent discovery has shown that, under certain conditions, RNA exhibits proteolytic activity.

C. NUCLEOPROTEIN

Both DNA and a specific type of RNA usually occur in cells as chemical complexes with proteins. It has been estimated that DNA accounts for only 30% of the liver nuclei chromatin

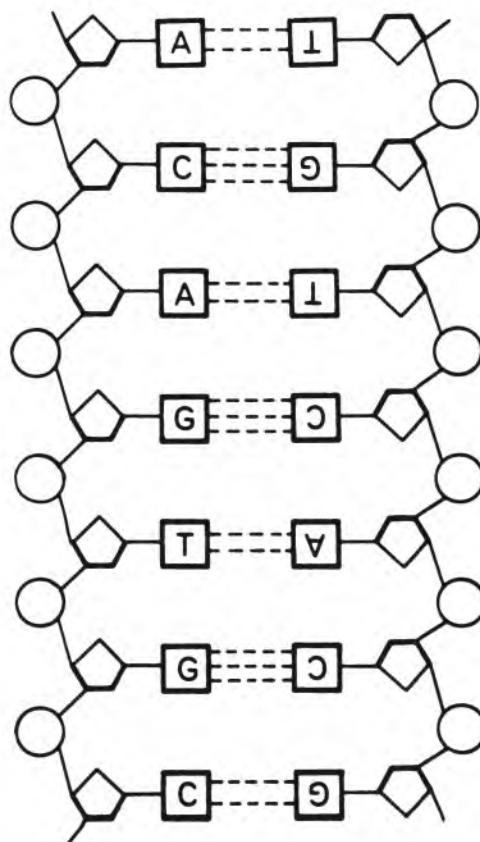


FIGURE 2.8. Diagrammatic representation of DNA structure. The circles represent phosphates; the pentagons, deoxyribose. The interrupted lines linking the bases (squares) indicate hydrogen bonds: three for G-C pairs, two for A-T pairs. (From Crick, F.H.S., *Sci. Am.*, 1957. With permission.)

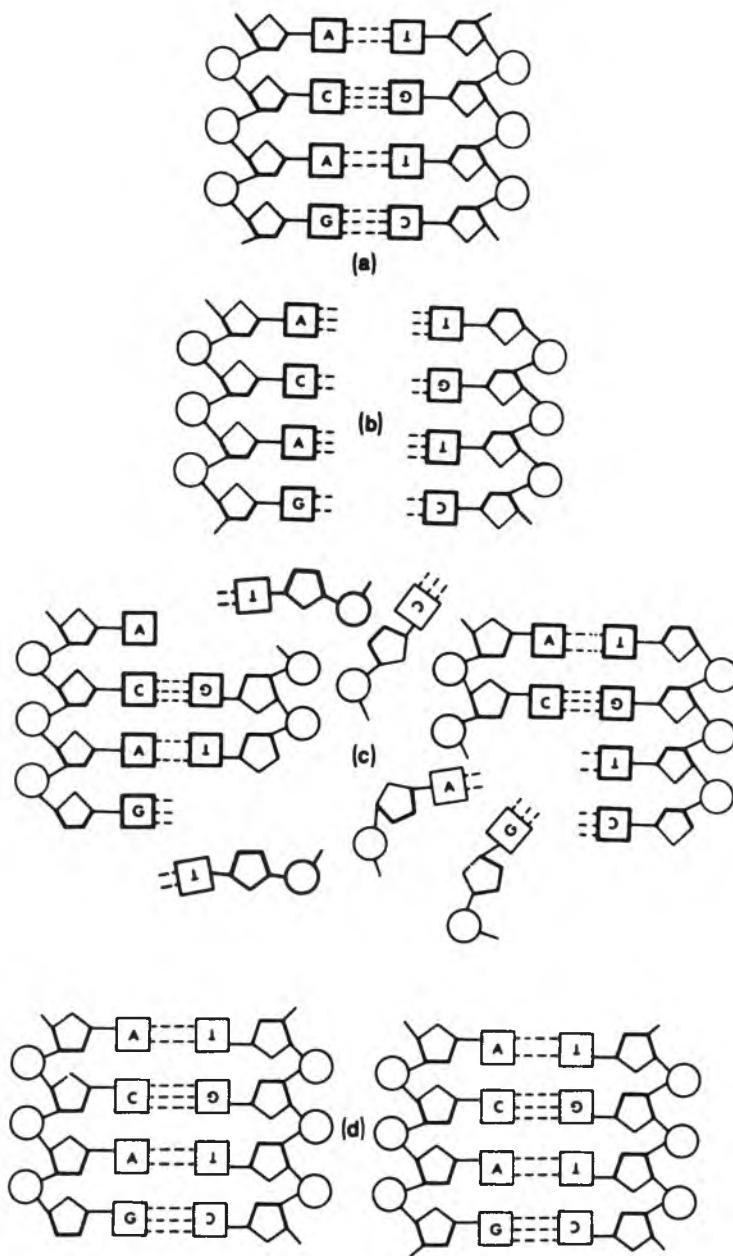


FIGURE 2.9. A schematic representation of DNA replication. The two strands of the DNA molecule (a) separate in the region undergoing replication (b). Free nucleotides pair with their appropriate partners and are linked together (c). The whole process proceeds in a zipper-like fashion until two complete DNA molecules are finally formed (d). The newly formed polynucleotide chains are indicated by shading. (From Crick, F.H.S., *Sci. Am.*, 1957. With permission.)

fraction, while the remainder includes histones, nonhistone (acidic protein) protein, and RNA. There are two basic proteins, protamine and histone, that complex with DNA. The DNA-protamine complex is found only in developing and mature spermatozoa. Histone exhibits much greater heterogeneity within the same nucleus than protamine. The extraction of the DNA-histone complex is always accompanied by extraction of DNA-bound acid protein.

There are two major types of histones, lysine-rich and arginine-rich. Like protamine, the histone is bound to DNA primarily by ionic bonds between the positively charged basic side chain and negatively charged phosphate group.

D. NONHISTONE PROTEIN

Histone has been considered as a genetic regulator. The addition of histone inhibits DNA and RNA synthesis *in vitro*. Lysine-rich histones are potent inhibitors of *in vitro* RNA synthesis, but arginine-rich histones inhibit only weakly. It is not yet clear whether this represents a specific regulatory capability or a relatively unspecific interference with the template activity of the primer DNA by the formation of insoluble nucleohistone complexes. Recently, it was discovered that a class of enzymes in calf thymus nuclei specifically acetylates histone. Acetylated histone inhibits RNA synthesis much less than nonacetylated histone. There is a significant percentage of DNA-linked protein that is not histone. In thymus, 27% of the DNA-linked protein is nonhistone.

E. BIOSYNTHESIS OF NUCLEIC ACID AND PROTEIN IN RELATION TO THE CELL CYCLE

DNA synthesis in a normal cell occurs only during the S phase of the cell cycle. However, several studies have shown that the incorporation of ³H-thymidine into DNA occurs not only in the S phase, but also in the G₁ and G₂ phases after a supralethal dose of X-radiation.²⁷ It has been suggested that the labeling pattern of cells in G₁ and G₂ with ³H-thymidine is an indication of a mechanism that actively repairs radiation damage. This is a good example in which radiation was utilized as a tool in understanding the potential function of a cell.

In HeLa cells, the rate of RNA synthesis is constant during G₁, and it approximately doubles during the first half of S; it then remains constant during the remainder of S and G₂.⁴² When DNA synthesis was blocked, the acceleration of RNA synthesis in the S phase depended upon the duplication of DNA. No RNA synthesis occurs in mitosis. During the early part of G₁, all the necessary enzymes for DNA synthesis (such as thymidine kinase and DNA polymerase) are induced, and by late G₁, they are fully formed. Tyrosine aminotransferase is synthesized by rat hepatoma tissue culture cells during all phases of the cell cycle, but can be induced by adrenal steroid hormones only during the latter portion of the G₁ phase and the entire S phase of the cell cycle.²⁴

In the crypts of the rat small intestine, cycloheximide prevents the entrance of cells from G₂ to M. Because cycloheximide inhibits protein synthesis, it is presumed that protein synthesis in G₂ is required for the normal progress of cells from G₂ to M.⁴² In the pea root tip meristem, entry of G₁ cells into S, G₂ cells into M, and the progression of G₁ cells through S → G₂ → M was prevented by carbohydrate starvation, anaerobiosis, or treatment with 2,4-dinitrophenol.⁴⁴ These data show that DNA synthesis and mitosis require aerobic conditions and are energy-dependent.

VII. REGULATION OF ENZYMES IN MAMMALIAN CELLS

The regulation of enzymes in mammalian cells is very complex and poorly understood. The activities of certain enzymes are regulated at the translation (cytoplasm) level rather than the transcription (nucleus) level.⁴⁰ It is now established that some hormones induce the synthesis of certain enzymes via increasing RNA synthesis. For example, hydrocortisone stimulates the liver enzyme tyrosine transaminase via increasing RNA synthesis. Estrogen increases protein and RNA synthesis in the rat uterus. Androgenic hormones also stimulate RNA and protein

synthesis in the liver. The mechanism of hormone action on the induction of enzyme synthesis is still unclear; however, in many cases the effect of the hormone is mediated by adenosine 3',5'-cyclic monophosphate (cyclic AMP), which is referred to as a second messenger.³⁹ Dibutyryl cyclic AMP ($\text{6N},\text{O}_2'$ -dibutyryl-adenosine-3',5'-cyclic monophosphate) induces the activity of tyrosine aminotransferase in rat hepatoma cells¹⁰ in culture and tyrosine hydroxylase in mouse neuroblastoma cells in culture.²⁹

VIII. REGULATION OF GROWTH AND DIFFERENTIATION OF MAMMALIAN CELLS IN CULTURE

Several studies have identified physiological substances that affect the growth and differentiation of mammalian tumor and normal cells in culture. These include cyclic nucleotides,^{13,15,26,29-31,45} growth factors,^{5a,19,38} and certain vitamins such as vitamin A,^{20,25} vitamin C,^{3,32} β -carotene,¹¹ vitamin D,⁶ and vitamin E.^{33,34} Because the growth rate and the degree of differentiation are linked with the radiosensitivity, the above agents may modify the radiosensitivity of mammalian tumor and/or normal cells. Unfortunately, very little work has been done on the modification of radiosensitivity by physiological substances. Among various kinases, protein kinase A (PKA), PKC, and protein-tyrosine kinase are important in the regulation of growth and differentiation of mammalian cells. They are known to phosphorylate specific proteins to control growth and differentiation of cells. These kinases can also interact with each other to regulate cell functions.^{14,22a} The importance of these kinases in radiosensitivity has not been adequately studied.

IX. ONCOGENES AND SIGNAL TRANSDUCTION

Oncogenes were first discovered as retrovirus-encoded genes that produced tumors in birds and rodents.^{5b} Homologous sequences of viral oncogenes were also found in mammalian cells. Transforming genes of ovarian tumor retroviruses contain a specific coding region called *v-myc*. The same region of a cellular gene is called *c-myc*. The transforming function of retroviral oncogenes depends primarily upon transcriptional activity rather than mutations in the coding region. This high transcriptional activity of retroviral oncogenes results from retroviral promoters. The *v-myc* by itself is sufficient to transform mammalian cells in culture, whereas *c-myc* does not.

The cellular genes and oncogenes encode proteins that play a key role in regulating cell proliferation, differentiation, and transformation.^{2,4,37a} Oncogenes can be divided into four classes: growth factors (e.g., *v-sis*), growth factor receptors (*v-erbB*, *v-fms*, *v-kit*), transducers of growth factor responses (*v-src*, *v-ras*, *v-raf*), and transcriptional factors that mediate growth factor-induced gene expression (*v-jun*, *v-fos*). Generally, the transcription factor-type oncogenes interact with oncogenes from other categories to regulate cell transformations.

In spite of the intensive studies on molecular carcinogenesis, the mechanisms of growth regulation, differentiation, and transformation remain poorly understood. This is due to the fact that these biological processes are regulated by a complex set of events with a certain amount of redundancy. The radiosensitivity of cells can be investigated following perturbation of oncogenes, growth factors, or pharmacological agents. In addition, the availability of cDNA probes for many cellular oncogenes, and monoclonal and polyclonal antibodies to several specific proteins provides new opportunities to investigate the mechanisms of action radiation on gene regulation.

X. IMMORTALIZATION OF MAMMALIAN CELLS

A number of plasmid and retroviral vectors carrying only the gene needed to immortalize cells from such viruses as SV40, polyoma, and adenoma type 5 have been constructed and used successfully to immortalize rodent and human fibroblast and epithelial cells.^{1,21} Recently, we have established immortalized cloned acinar cells from adult rat parotid glands³⁶ and dopamine-producing cells from rat fetal mesencephalic tissue^{37a} by transfecting them with plasmid vectors, pSV₃^{neo} and pSV₅^{neo} carrying T-antigen genes from SV40, and polyoma viruses, respectively. We have also established human nontumorigenic and tumorigenic cell lines from pleomorphic adenomas.³⁵ These cell lines can be very valuable in the study of radiosensitivity and carcinogenesis of mammalian cells *in vitro*.

XI. SUMMARY AND COMMENTS

During the last decade, considerable progress has been made in understanding the structure and function of cells. The technique of examining cells under the electron microscope has improved markedly, and there is no doubt that more refined methods will be developed in the future. In addition to this, rapid progress has been made in the technique of isolation, purification, structural analysis, and synthesis of a given macromolecule. Such studies have provided a better understanding of the regulation of biosynthesis of DNA, RNA, and protein in the cell. Considerable work is being done to elucidate the role of hormones and growth factors in the regulation of protein synthesis. The technique of obtaining synchronized cells *in vitro* has proved very useful in the understanding of the action of several physical and chemical agents on the cell. Although some work on the synthesis of DNA, RNA, and protein in different phases of the cell cycle has been done, a detailed understanding of molecular events during the movements of cells through the cycle is lacking. In addition, the subcellular changes during the movements of cells from one phase to another are unknown. The correlation of structural changes with biochemical ones during movements of cells from one phase to another would provide a better knowledge of cell function. Recently, several physiological substances that affect the growth rate, morphology, and differentiation of mammalian normal and tumor cells have been identified. These include cyclic nucleotides, growth factors, and certain vitamins (vitamin A, β-carotene, vitamin C, vitamin D, and vitamin E). These agents are potentially very important in the modification of radiation damage in mammalian normal and tumor cells. In addition, the availability of oncogene probes, specific antibodies, and new human cell lines provides a new opportunity to study the mechanisms of radiosensitivity and their modifications by pharmacological and physiological agents.

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Chapter 3

PHYSICS OF RADIATION BIOLOGY

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I. INTRODUCTION

To better understand the effects of radiation on the biological system, it is necessary to understand the fundamental principles of atomic and nuclear physics. Therefore, this chapter will describe only those concepts of physics that are essential for an understanding of radiation biology. These principles will be discussed in a broad and conceptual form. Some of the important references on the subject are listed at the end of this chapter.¹⁻⁵

II. STRUCTURE OF AN ATOM

An atom consists of a central mass called the *nucleus*, which is surrounded by orbital *electrons* (Figure 3.1). The atom has a diameter of about 10^{-8} cm, whereas the nucleus has a diameter of about 10^{-12} cm.

A. ELECTRON

Electrons are negatively charged particles and orbit the atomic nucleus in a precisely defined path, each path being characterized by its own unique energy level. Electrons are positioned in shells or energy levels that surround the nucleus. The first or K shell contains no more than 2 electrons, the second or L shell no more than 8 electrons, and the third or M shell no more than 18 electrons (Figure 3.2). The outermost electron shell of an atom, no matter which shell it is, never contains more than 8 electrons. Electrons in the outermost shell are termed *valence electrons* and determine to a large degree the chemical properties of an atom. An atom with an outer shell filled with electrons seldom reacts chemically. These atoms constitute elements known as the inert gases (helium, neon, argon, krypton, xenon, and radon).

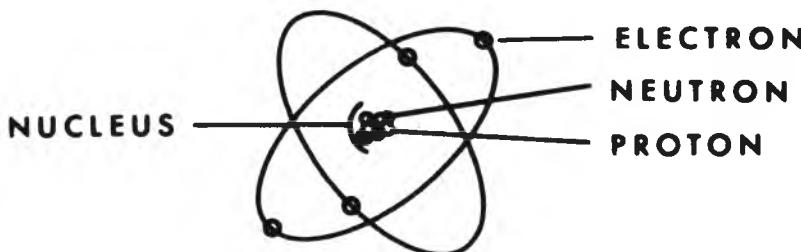


FIGURE 3.1. Schematic representation of the structure of an atom.

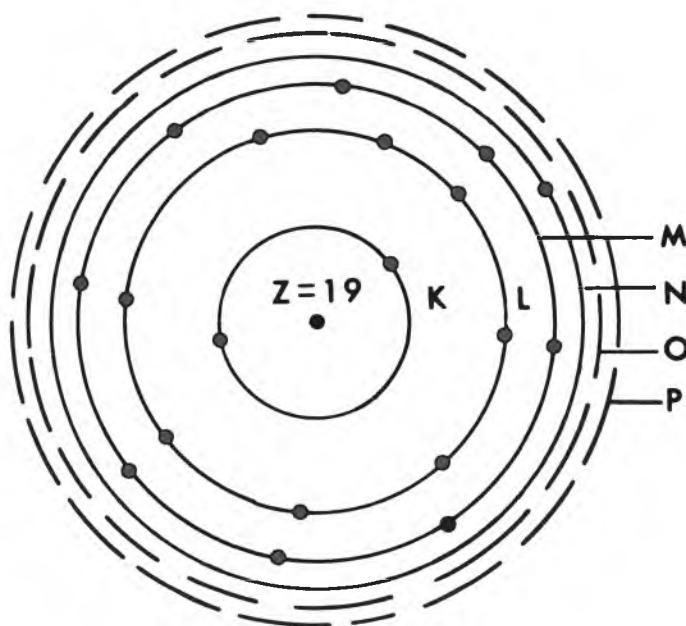


FIGURE 3.2. Electron orbits in potassium ($Z=19$). For simplicity, the orbits are represented in circular form. (From Hendee, W.R., *Medical Radiation Physics*, Year Book Medical, Chicago, 1970, 21. With permission.)

The energy required to completely remove an electron from an atom is termed the *binding energy* (E_B). Binding energies are negative because they represent amounts of energy that must be supplied to remove electrons from atoms. Electron shells often are described in terms of the binding energy of the electrons occupying the shell. For example:

$$E_B \text{ for a hydrogen K shell electron} = 13.5 \text{ eV}$$

$$E_B \text{ for a hydrogen L shell electron} = 3.4 \text{ eV}$$

$$\text{Energy required to move an electron from the K to the L shell} = (-3.4 \text{ eV}) - (-13.5 \text{ eV}) = 10.1 \text{ eV.}$$

Electrons lose or gain energy only when they jump from one orbit to another. No change in energy occurs so long as the electrons remain in a specified orbit. Vacancies, or "holes," exist in electron shells from which electrons have been removed. The vacancies are filled promptly by electrons cascading from energy levels farther from the nucleus. As the vacancies are filled, energy is released — usually in the form of electromagnetic radiation. During the transition of a particular electron, the energy released equals the difference in binding energy between the original and the final energy level for the electron. In most cases, the energy is released as a "photon," or packet of electromagnetic radiation. Occasionally, the energy may be used to eject a second electron, usually from the same shell as the cascading electrons. The ejected electron is termed an *Auger electron*. Electromagnetic radiation released during electron transition is termed *characteristic radiation*, because the photon energies are characteristic of differences in the binding energy of electrons in a specific atom.

B. NUCLEUS

The nucleus of the atom is composed of two particles, protons, and neutrons, referred to collectively as *nucleons*. Each proton has a positive charge of 1.6×10^{-19} coulomb (C), equal

in magnitude but opposite in sign to the charge of an electron. The mass of a proton is 1.6724×10^{-27} kg. The neutron has no charge but has a mass of 1.6747×10^{-27} kg. The number of protons in the nucleus is the atomic number (Z) for the atom, whereas the total number of protons and neutrons in the nucleus is the mass number (A). Isotopes of an element have the same atomic number but a different mass number. The atomic mass unit (amu) is a more convenient unit of mass for atomic particles: 1 amu is defined as 1/12 the mass of the carbon nucleus with six protons and six neutrons.

$$1 \text{ amu} = 1.6605 \times 10^{-27} \text{ kg}$$

Masses of atomic particles, in amu, are

$$\text{Electron} = 0.00055 \text{ amu}$$

$$\text{Proton} = 1.00727 \text{ amu}$$

$$\text{Neutron} = 1.00866 \text{ amu}$$

The shell model of the nucleus was introduced to explain the existence of discrete nuclear energy states. In this model, nucleons are arranged in shells similar to those available to electrons in the extranuclear structure of an atom. Nuclei are stable if they contain 2, 8, 20, 82, or 126 protons, or similar numbers of neutrons. These numbers are termed the *magic number* and may reflect full occupancy of nuclear shells. Nuclei with odd numbers of neutrons or protons tend to be less stable than nuclei with even numbers of neutrons or protons. Apparently, the pairing of similar nucleons increases the stability of the nucleus as shown by the data tabulated below.

Number of protons	Number of neutrons	Number of stable nuclei
Even	Even	165
Even	Odd	57
Odd	Even	53
Odd	Odd	6

The number of neutrons is about equal to the number of protons in stable nuclei with low atomic numbers. As the atomic number increases, the number of neutrons in stable nuclei increases more rapidly than the number of protons. Therefore, nuclei with intermediate or high atomic numbers contain a neutron:proton ratio (*n/p*) greater than one. However, if the *n/p* is much greater than one, nuclei may become unstable. The unstable nuclei emit radiation of different energy.

Electrostatic repulsive forces exist between particles of similar charge. Because the distance between protons is less than the diameter of the nucleus, the protons remain together due to the existence of a *nuclear force*. This force is stronger than the electrostatic repulsive force and binds neutrons and protons together within their nucleus. The nuclear force is effective only when nucleons are separated by a distance smaller than the diameter of the nucleus.

The mass of a nucleus is less than the sum of masses of the nucleons in the nucleus. The mass difference is termed the *mass defect* and represents an amount of energy that must be supplied to separate the nucleus into individual nucleons. This amount of energy is the *binding energy of the nucleus*. The relationship between mass and energy is described by Einstein's formula:

$$E = mc^2$$

In this equation, E represents an amount of energy equivalent to a mass m, and c is a conversion coefficient equal to the speed of light *in vacuo* (3×10^8 m/sec). The binding energy of the carbon nucleus with six protons and six neutrons ($^{12}_6\text{C}$) is calculated as follows:

$$\text{Mass 6 protons} = 6(1.00727 \text{ amu}) = 6.04362 \text{ amu}$$

$$\text{Mass 6 neutrons} = 6(1.00866 \text{ amu}) = 6.05196 \text{ amu}$$

$$\text{Mass 12 nucleons of } ^{12}_6\text{C} = 12.09558 \text{ amu}$$

$$\text{Mass } ^{12}_6\text{C nucleus} = 12.00000 \text{ amu}$$

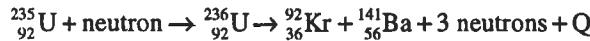
$$\text{Mass defect} = (\text{mass of nucleons} - \text{mass of nucleus}) = 0.09558 \text{ amu}$$

$$\text{Binding energy (}E_B\text{) of } ^{12}_6\text{C nucleus} = (0.09558 \text{ amu}) \times 931 \text{ MeV/amu} = 89.0 \text{ MeV}$$

$$\text{Average } E_B/\text{nucleon} = 89.0 \text{ MeV}/12 \text{ nucleons} = 7.42 \text{ MeV/nucleon}$$

III. NUCLEAR FISSION

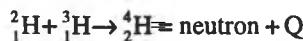
Energy is released if a nucleus with a high mass number separates, or *fissions*, into two parts — each with an average binding energy per nucleon greater than that of the original nucleus. Certain high-mass-number nuclei (e.g., ^{235}U , ^{239}Pu , and ^{233}U) fission spontaneously after absorbing a slowly moving neutron. For ^{235}U , a typical fission reaction is:



The energy released is designated as Q and averages above 200 MeV/fission. The energy is liberated primarily as gamma radiation and kinetic energy of fission products and neutrons. Products such as ^{92}Kr and ^{141}Ba are termed *fission by-products* and are radioactive. Many different by-products are produced during fission. Neutrons released during fission may interact with other ^{235}U nuclei, creating the possibility of a chain reaction, provided that a sufficient mass (critical mass) of fissionable material is contained in a small volume. The rate at which a material fissions may be regulated by controlling the number of neutrons available each instant to interact with fissionable nuclei. Fission reactions within a nuclear reactor are controlled in this way. Uncontrolled nuclear fission results in an *atomic explosion*.

IV. NUCLEAR FUSION

Certain low-mass nuclei may be combined to produce one nucleus with an average binding energy per nucleon greater than that for either of the original nuclei. This process is called *nuclear fusion* and is accompanied by the release of large amounts of energy. For example:



In this case Q = 18 MeV. Nuclei moving at very high velocities possess enough momentum to overcome the repulsive force of the nuclei. Adequate velocity may be attained by heating a sample containing low-atomic-number nuclei to a temperature greater than 12×10^6 K, roughly equivalent to the temperature in the inner region of the sun. Temperatures this high are attained on earth only in the center of a fission explosion. Consequently, a fusion (hydrogen) bomb must be “triggered” with an atomic bomb.

V. NUCLEAR NOMENCLATURE

The differences between isotopes, isotones, and isobars are illustrated as follows:

	Atomic Number (Z)	Neutron Number (N)	Mass Number (A)	Examples
Isotopes	Same	Different	Different	Isotopes of carbon are ^{12}C , ^{10}C , ^{14}C , ^{13}C , ^{15}C
Isotones	Different	Same	Different	^{3}He , ^{7}Li , ^{7}Be , ^{8}B
Isobars	Different	Different	Same	^{3}He , ^{7}Li , ^{8}B
Isomers	Same	Same	Same, but different nuclear energy state	

An isotope is specified by its chemical symbol together with its mass number as a left superscript. An isotope is often called a *nuclide*.

VI. RADIOACTIVE DECAY

With the exception of $^{209}_{83}\text{Bi}$, each nucleus with an atomic number greater than 82 is unstable. Many nuclei with atomic numbers less than 82 are unstable. By 1965, 259 stable and 1130 unstable nuclides had been identified. Unstable nuclei undergo transitions such as nuclear fission or, more frequently, radioactive decay. Energy is released during this transition. The types of radioactive decays are described below.

A. ALPHA

Many nuclei with high atomic numbers decay by alpha emission. For example:

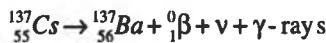


The total energy released during the radioactive decay of a nucleus is termed the *transition energy*. During decay, energy is released as kinetic energy of the alpha particles. Energy also may be released as gamma radiation. Alpha particles from a specific nuclide are ejected with discrete energies. The decay scheme of ^{226}Ra is shown in Figure 3.3. A 4.78-MeV α -particle is released during 94% of ^{226}Ra transition. In the remaining transitions, a 4.59-MeV α is accompanied by γ -rays of 0.19 MeV. The percent of nuclei that decay by a particular path is termed the *branching ratio* for the path. The transition energy (44.78 MeV) is the same for all transitions of ^{226}Ra nuclei.

B. NEGATRON

In 1896, Henri Becquerel discovered the emission of energetic electrons from uranium salts. This mode of decay is usually called *beta decay*. Beta decay also refers to the emission of positive electrons from certain nuclei. To distinguish these decay processes, negative electron decay should be referred to as *negatron decay*, whereas positive electron decay is *positron decay*.

The ratio of neutrons to protons (n/p) in a negatron-emitting nuclide is greater than that required for maximum stability of the nucleus. Negatron decay results in an increase in atomic number by one and a constant mass number. Negatron transition is illustrated below.



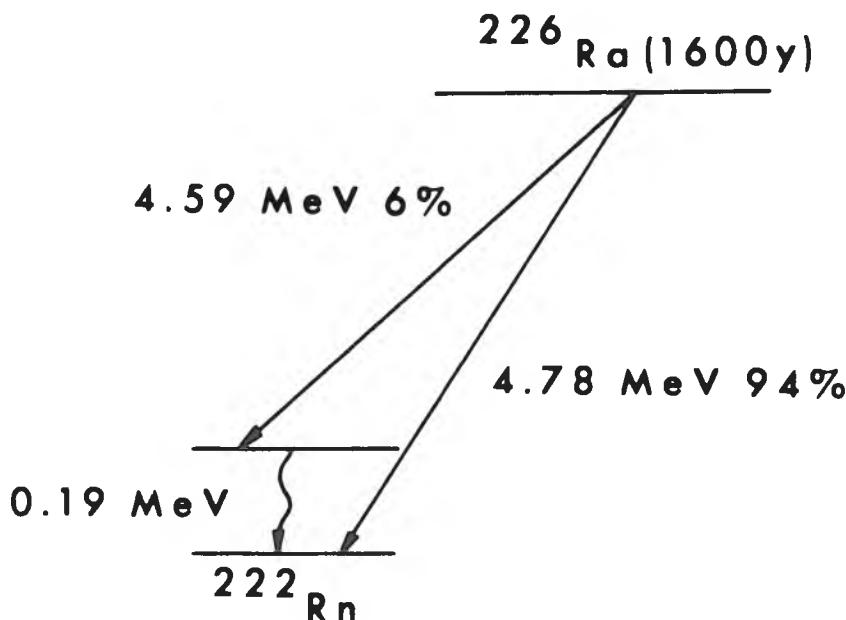
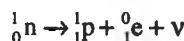
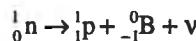


FIGURE 3.3. Radioactive decay scheme for alpha decay of ^{226}Ra .

During the above transition, one neutron is transformed into one proton. In addition, neutrino and γ -rays are released. The transition may be written:



or



A decay scheme for ^{137}Cs is presented in Figure 3.4. A negatron with a maximum energy of 1.17 MeV is released during 5% of all decays of ^{137}Cs . In the remaining 95%, a negatron with $E_{\max} = 0.51$ MeV is accompanied by γ -rays of 0.66 MeV or by an electron ejected by internal conversion.

1. Spectrum of Negatron Decay

Although negatrons emitted by a particular nuclide have discrete maximum energies, most negatrons are ejected with energy lower than these maxima. The mean energy E_{mean} of negatrons emitted during a particular transition is approximately $E_{\max}/3$. The shape of the negatron spectrum and the value for E_{\max} are characteristic of the particular nuclide. The spectrum of ^{32}P , which emits negatrons with a single E_{\max} , is shown in Figure 3.5.

C. NEUTRINO

During the decay of a particular nucleus, the transition energy already exceeds the sum of the energy as gamma radiation and the kinetic energy of the ejected electrons. The energy unaccounted for during each transition is possessed by the second particles, termed the *neutrino* (ν).

$$\text{Energy of neutrino} = E_{\max} - \text{kinetic energy of electron}$$

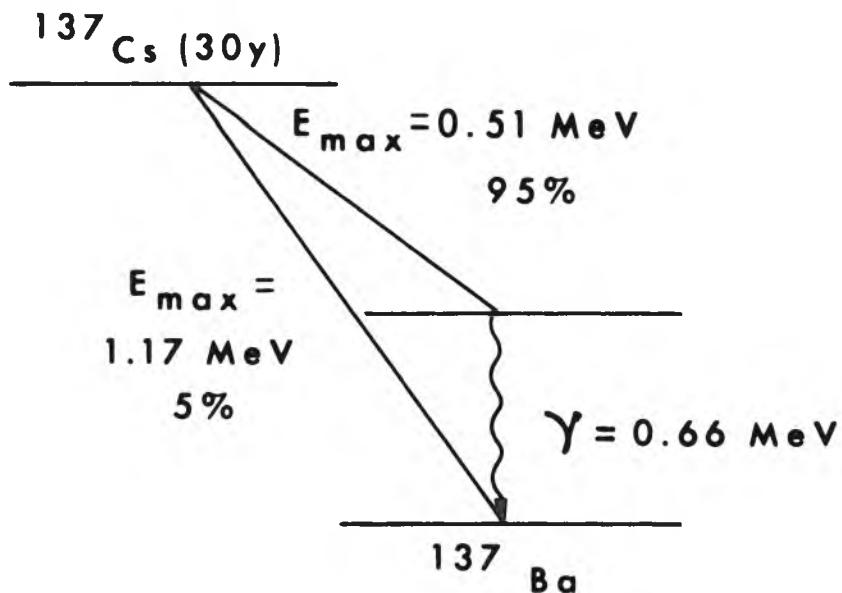


FIGURE 3.4. Radioactive decay scheme for negatron decay of ^{137}Cs .

Neutrinos are uncharged particles with undetectable small mass. There are two forms of neutrinos. One form (ν) describes particles ejected during positron decay. The second form, called the antineutrino ($\bar{\nu}$), is released during negatron decay.

D. GAMMA EMISSION

During radioactive decay, the daughter nucleus is formed in an excited and unstable state. A γ -ray is released during transition from the excited state to the ground energy level. Gamma

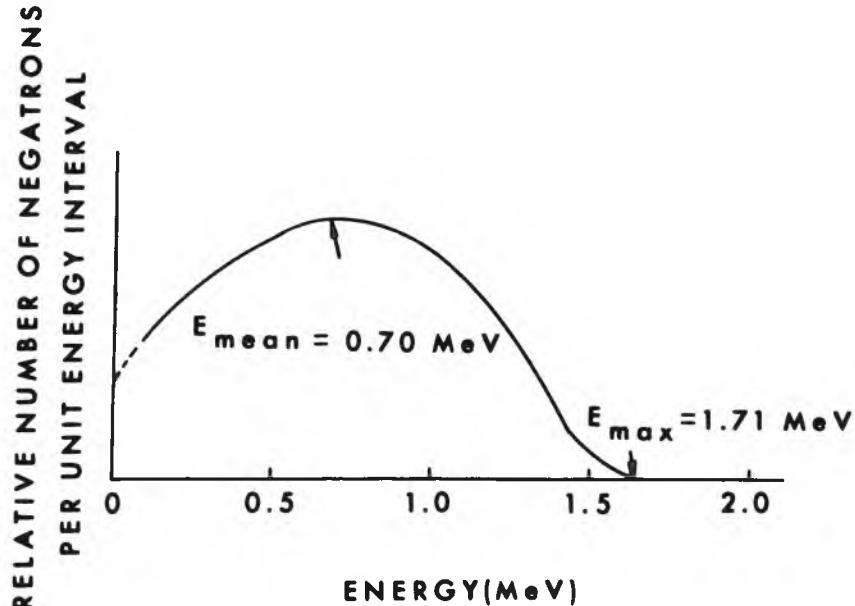
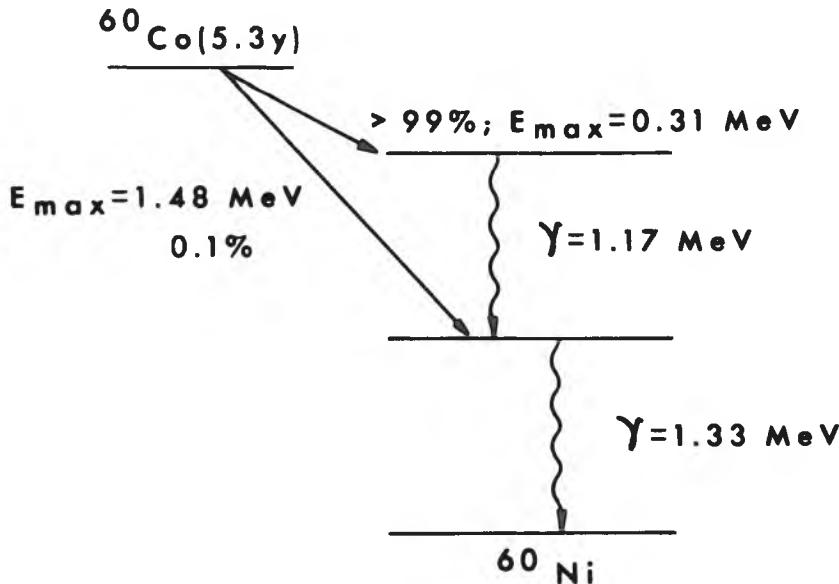


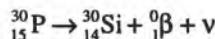
FIGURE 3.5. Energy spectrum for negatrons from ^{32}P . (From Hendee, W.R., *Medical Radiation Physics*, Year Book Medical, Chicago, 1970, 35. With permission.)

FIGURE 3.6. Radioactive decay scheme for ^{60}Co .

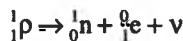
rays and X-rays occupy the same region within the electromagnetic spectrum and are distinguishable only by their origin; γ -rays result from nuclear transition, and X-rays result from interactions of electrons outside the nucleus. Every gamma transition is preceded by either electron capture or by emission of an α particle, negatron, or positron. Nuclides emit X-rays with characteristic energies. Figure 3.6 shows a decay scheme of ^{60}Co which emits two γ -rays of 1.17 and 1.33 MeV.

E. POSITRON DECAY AND ELECTRON CAPTURE

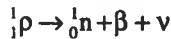
Positron-emitting nuclides possess n/p ratios lower than those required for maximum stability. Positron decay is accompanied by a decrease in the atomic number by one, and by no change in mass number. A representative positron transition is:



During the above transition, one proton is transformed to one neutron. In addition, neutrino is released. The transition may be written:

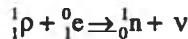


or



The positron is unstable. On close approach to a negatron, each will annihilate the other, and their mass appears as electromagnetic radiation — usually two 0.5-MeV photons moving in opposite directions.

The nuclei that cannot furnish at least 1.02 MeV for the transition do not decay by positron emission. These nuclei increase their n/p ratio by *electron capture*. Most electrons are captured from the K shell, although electrons may be captured from other shells. During electron capture, the nuclear transition may be written:



During electron capture, a hole is created in an electron shell deep within the atom. This vacancy is filled by an electron cascading from an energy level farther from the nucleus. Energy released during this transition appears as X-radiation or as the kinetic energy of an Auger electron.

Nuclei with transition energies greater than 1.02 MeV may decay by both positron emission and electron capture. The electron capture branching ratio is 10% for ^{22}Na ; therefore, 90% of all decay occurs with the emission of a positron.

F. INTERNAL CONVERSION

In some disintegrations, the excited nuclei may get rid of its excess energy by internal conversion. In this process, γ -rays from the nucleus interact with one of its own inner electrons, which is ejected with a kinetic energy equal to the energy of the γ -ray minus the binding energy of electrons. During internal conversion, no γ -ray is emitted; however, characteristic X-radiation and Auger electrons are produced when the ejected conversion electron is replaced. The probability of internal conversion increases rapidly with atomic number and with the lifetime of the excited state of the nucleus.

The rate of decay is referred to as the activity of the sample. The unit of radioactivity is the curie (Ci).

G. MATHEMATICS OF RADIOACTIVE DECAY

$$1 \text{ Ci} = 3.7 \times 10^{10} \text{ disintegrations per second (dps)}$$

$$1 \text{ mCi} = 3.7 \times 10^7 \text{ dps}$$

$$1 \text{ } \mu\text{Ci} = 3.7 \times 10^4 \text{ dps}$$

The equation for radioactive decay is:

$$\frac{N}{N_0} = e^{-\lambda t}$$

N_0 is the initial number of radioactive atoms at the time $t = 0$. N is the number of radioactive atoms left after time t ; λ is a decay constant. The physical half-life ($T_{1/2}$) of a radioactive nuclide is the time required to reduce the initial activity to half. $N = 1/2 N_0$ when $t = T_{1/2}$ assuming $N = N_0$ when $t = 0$.

$$\frac{1N}{2N_0} = \exp(-\lambda T_{1/2})$$

$$\frac{1}{2} = \exp(-\lambda T_{1/2})$$

$$2 = \exp(\lambda T_{1/2})$$

$$\ln 2 = \ln(\exp \lambda T_{1/2})$$

$$\ln 2 = \lambda T_{1/2}$$

$$T_{1/2} = \frac{\ln 2}{\lambda} = \frac{0.693}{\lambda} \quad (\ln 2 = 0.693)$$

$$\lambda = \frac{\ln 2}{T_{1/2}} = \frac{0.693}{T_{1/2}}$$

Every radioactive nuclide exhibits a characteristic decay constant and physical half-life. Specific activity is defined as the activity per unit mass of a radioactive sample.

H. PRODUCTION OF X-RAYS

In 1895, Roentgen discovered a new type of radiation that he called the X-ray because of its unknown nature. One of the characteristic features of the new radiation was its ability to penetrate even solid matter. Medical fluoroscopy and radiography were the outgrowths of Roentgen's observation: "If the hand is held before the fluorescent screen, the shadow shows the bones darkly, with only faint outlines of the surrounding tissues."

All modern X-ray tubes are known as *Coolidge* tubes after W.C. Coolidge, who introduced the basic design in 1913. In the Coolidge tube, electrons supplied by an electrically heated filament are accelerated by a high positive potential to a target (anode). The target is a metal of high melting point and high atomic number. Modern X-ray tubes are evacuated to a high degree. During X-ray production, large amounts of heat are generated in the target, which is made hollow to permit cool water or oil to be circulated through it. When an electron interacts with the target nucleus, part of its energy is degraded to heat, and part goes into producing X-rays; the exact energy division depends upon the details of the collision at the target, the energy of the electron, and the atomic number of the target material. At 100 keV, less than 1% of the energy of the primary electron is converted into radiation, and over 99% appears as heat. At high energies — especially in materials of high atomic number, such as tungsten or lead — the major portion of the energy of the primary electrons is converted into radiation and little into heat. The energy of the X-ray beam depends upon the accelerating voltage; a higher voltage produces an X-ray of greater energy.

The special distribution of an X-ray beam can be determined with an X-ray spectrometer. In general, an X-ray spectrum (Figure 3.7) is made up of two components: (1) the continuous distribution of radiation from lower to the maximum energy is called *bremstrahlung* or *white* radiation, and (2) characteristic peaks in the X-ray spectrum reflect the emission of characteristic X-rays produced as electrons fill vacancies in the K shell of the tungsten atoms.

VII. INTERACTION OF PARTICULATE RADIATION

An interaction is elastic if the sum of the kinetic energies of the interacting entities is unchanged by the interaction. If the sum of the kinetic energies is changed, then the interaction is inelastic. Protons (${}_1^1H$ nuclei), deuterons (${}_2^2H$ nuclei), α -particles, (${}_2^4He$ nuclei), and other heavy particles lose most of their energy when they interact inelastically with electrons of the absorbing materials. The transfer of energy is accomplished by interacting electrical fields, and physical contact is not required between the incident particles and the absorbing electrons. Part of the energy lost by incident particles is used to raise electrons in the absorber to energy levels farther from the nucleus. This process is termed *excitation*. Sometimes electrons are ejected from their atoms — a process known as *ionization*. Electrons ejected from atoms by incident radiation are referred to as *primary electrons*. Some primary electrons have enough kinetic energy to produce an additional ion pair as they migrate from their site of release.

Electrons ejected during the interaction of primary electrons are termed *secondary electrons*. Delta (δ)-rays are tracts of primary and secondary electrons in photographic emulsions exposed to ionizing radiation.

Energy transferred to an electron in excess of its binding energy appears as the kinetic energy of the ejected electrons. An ejected electron and the residual positive ions constitute an ion pair. An average energy of about 35 eV, termed W, is expended by heavy, positively charged particles per ion pair produced in air. The average energy required to remove an electron from nitrogen or oxygen is much less than 35 eV. On the average, 2.2 atoms are excited per ion pair produced in air.

The *specific ionization* (SI) is the number of primary and secondary ion pairs produced per unit length of path of the incident radiation. The specific ionization of α particles in air varies from about 30,000 to 70,000 ion pairs per centimeter. The specific ionization of protons and deuterons is slightly less than that for α particles. The *linear energy transfer* (LET) is the average loss in energy per unit length of path of the incident radiation and is expressed as keV/ μm or erg/ μm . LET depends upon the mass, charge, and velocity of the particles. A particle with greater mass and charge, but with lower velocity, will have the higher LET.

$$\text{LET} = \text{SI} \times \text{W}$$

The *range* of ionizing particles in a particular medium is the straight-line distance traversed by the particles with energy E. The range in a particular medium may be estimated from the average LET.

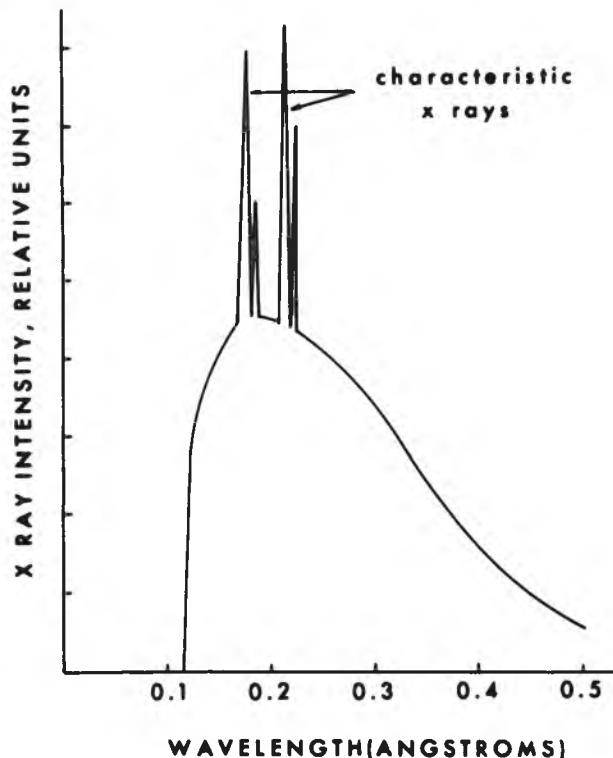


FIGURE 3.7. X-ray spectrum illustrating the contribution of characteristic X-rays produced as electrons fill holes in the K shell of tungsten. (From Hendee, W.R., *Medical Radiation Physics*, Year Book Medical, Chicago, 1970, 71. With permission.)

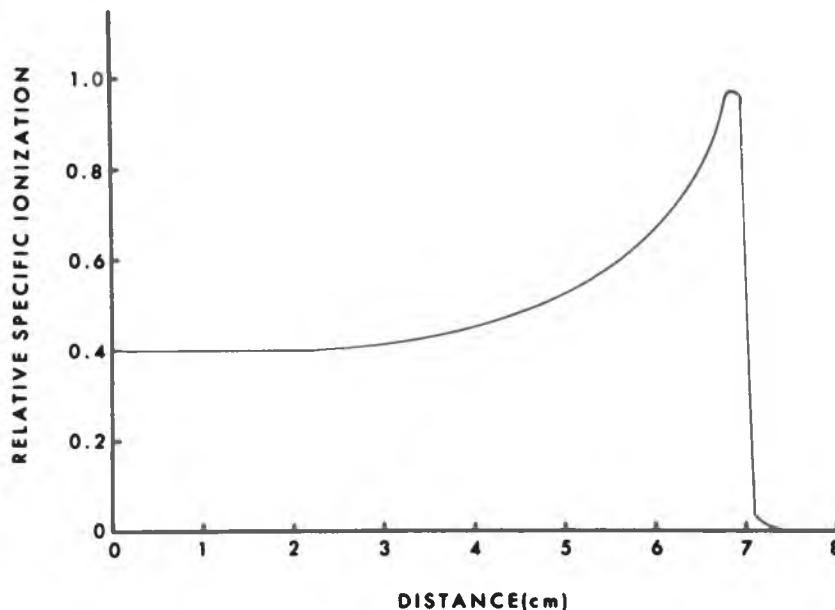


FIGURE 3.8. The relative specific ionization of 7.7-MeV α particles from the decay of ^{214}Po , plotted as a function of the distance transversed in air. (From Hendee, W.R., *Medical Radiation Physics*, Year Book Medical, Chicago, 1970, 58. With permission.)

$$\text{Range} = \frac{E}{\text{LET}}$$

The SI and LET are not constant along the entire path of monoenergetic charged particles traversing a homogeneous medium. As the particles slow down, the SI increases, because nearby atoms are influenced for a longer period. The region of the increased specific ionization is called the "Bragg peak." Finally, the particles capture two electrons, become neutral, and can no longer ionize; therefore, the rate of ionization falls abruptly. The specific ionization of 7.7-MeV alphas from ^{214}Po is shown in Figure 3.8.

The range of particle radiation depends upon the energy, mass, charge, and the atomic number of the medium through which the particles are passing. For the same energy, α particles have less range than deuterons, protons, or electrons. Protons and neutrons have similar mass; however, for the same energy, neutrons have greater range than protons, because they have no charge.

An intense beam of high-energy negative π mesons became available at Los Alamos at the end of 1972. These particles have a mass of the electrons and are produced by bombarding a target with particles (protons and deuterons) accelerated to very high energies. Superimposed upon the Bragg peak for these particles is the energy released as slowly moving π mesons interact with nuclei of the absorbing medium, causing these nuclei to undergo spallation and produce "stars" (Figure 3.9).

A. INTERACTIONS OF ELECTRONS

Interactions of negative and positive electrons may be divided into three categories: (1) scattering by electrons, (2) elastic scattering by nuclei, and (3) inelastic scattering by nuclei.

1. *Scattering by electrons:* Negative and positive electrons traversing an absorbing medium transfer energy to electrons of the medium. Incident electrons lose energy and are

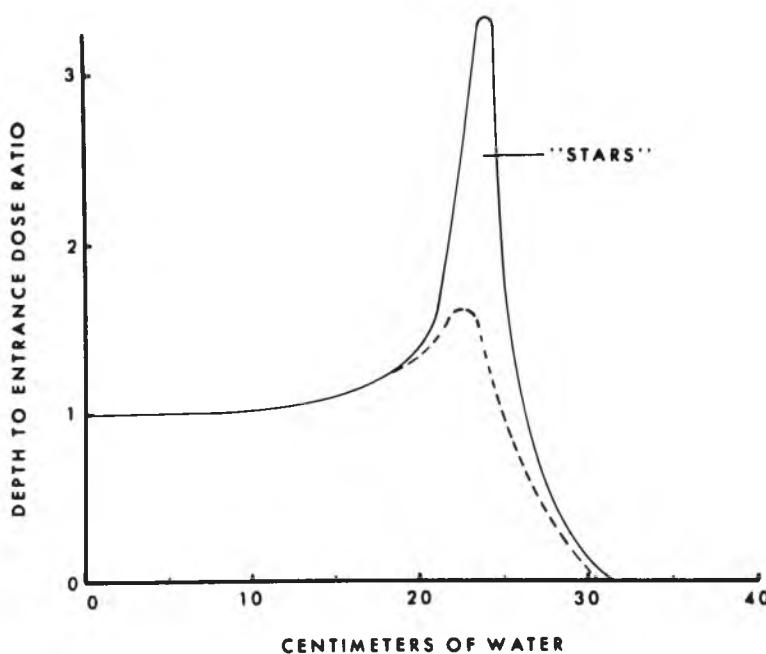


FIGURE 3.9. Ratio of the specific ionization (or "dose") at depth to that on the surface for a "pure" beam of negative π mesons with an energy of 96 MeV at the surface. The shaded area depicts the contribution of nuclear fragments produced near the end of the path of particles. (From Curtis, S. and Raju, M.A., *Radiat. Res.*, 34, 239, 1968. With permission.)

deflected at some angle with respect to their original direction. An electron receiving energy may be raised to an electron shell farther from the nucleus or may be ejected from the atom. The kinetic energy (E_k) of an ejected electron equals the energy (E) received minus the binding energy (E_B) of the electron.

$$E_k = E - E_B$$

The probability of electron-electron scattering increases with the atomic number of the absorber and decreases rapidly with increasing kinetic energy of the incident particles.

2. *Elastic scattering by nuclei:* Electrons are deflected with reduced energy during elastic interactions with nuclei of an absorbing medium. The probability of elastic interactions with nuclei varies with Z^2 (atomic number = Z) of the absorber and approximately with $1/E_k^2$, where E_k represents the kinetic energy of the incident electrons. The probability for elastic scattering by nuclei is slightly less for positrons than for negatrons with the same kinetic energy. Backscattering of negatrons and positrons in a radioactive sample is due primarily to elastic scattering by nuclei.
3. *Inelastic scattering by nuclei:* A negative or positive electron passing near a nucleus may be deflected with reduced velocity. The interaction is inelastic if energy is released as electromagnetic radiation during the encounter. A sudden deceleration, or "braking," of electrons gives rise to *bremsstrahlung* (the German term for braking radiation). The probability of bremsstrahlung production varies with Z^2 of the absorbing medium.

B. CERENKOV RADIATION

Visible light is radiated by charged particles moving through a medium at a velocity exceeding the velocity of light in the medium. The visible light is named Cerenkov radiation. Only a small fraction of the kinetic energy of high-energy electrons is lost by production of Cerenkov radiation.

C. INTERACTION OF NEUTRONS

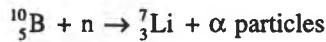
Slow (0 to 0.1 keV), intermediate (0.1 to 20 keV), fast (20 keV to 10 MeV), and high-energy neutrons are produced by the nuclear reactor. Neutrons with various kinetic energies are emitted by ^{252}Cf , a nucleus that fissions spontaneously. Neutron beams are available from neutron generators and cyclotrons, in which low-Z nuclei (e.g., ^3H or ^9Be) are bombarded by positively charged particles (e.g., nuclei of ^1H , or ^2H , or ^4He) moving at high velocities.

Neutrons lose energy either by elastic or inelastic collision with the nuclei of the absorbing material. The probability of elastic collision is greatest with nuclei of similar mass. Therefore, in biological tissue during elastic collision neutrons transfer most of their energy to hydrogen nuclei; recoil protons, which result from this interaction, produce ionization like any heavy, charged particles.

For neutrons with kinetic energy above 10 MeV, inelastic scattering also contributes to the energy lost in tissue. For example, inelastic interactions account for 30% of the energy deposited in tissues by 14.1-MeV neutrons. Most inelastic scattering occur with nuclei other than hydrogen. Energetic charged particles (e.g., proton or α particles) often are ejected from nuclei excited by inelastic interactions.

D. NEUTRON CAPTURE

Certain materials (e.g., lithium, boron, cadmium, and uranium) exhibit a high cross-section for the capture of slow neutrons. Energetic positively charged particles may be ejected by certain nuclei (e.g., ^6Li and ^{10}B) which capture neutrons. For example:



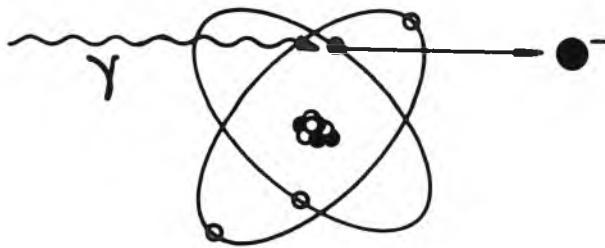
Other nuclei such as ^{235}U and ^{239}Pu fission spontaneously after absorbing a neutron.

VIII. INTERACTION OF X-AND GAMMA-RAYS

Gamma- and X-ray photons are attenuated (absorbed or scattered) in many ways as they traverse the medium. Of the various alternating processes, *photoelectric* and *Compton interactions* are the most important for radiation biologists. Interactions of less importance include coherent scattering, pair production, and photodisintegration. The rate at which X- or γ -ray photons are attenuated is a function of photon energy as well as of absorbing materials.

A. THE PHOTOELECTRIC EFFECT

When an X- or γ -ray photon collides with an atom, it may transfer all its energy to an orbital electron, which is ejected out with a kinetic energy. The process of energy absorption is called the *photoelectric effect*, and ejected electrons are known as photoelectrons. Kinetic energy of ejected electrons (E_k) equals the energy of the incident photon ($h\nu$) minus the binding energy of the electron (E_B). Thus, the photoelectric effect involves bound electrons whose ejection probability is maximum if the photon has just enough energy to knock the electron from its shell. The photoelectric cross-section varies with energy approximately as $1/E^3$. The photo-



$$\text{Energy of photoelectron} = \\ \text{energy of photon} - \\ \text{binding energy (B.E.) of electron}$$

FIGURE 3.10. Schematic representation of photoelectric absorption of a photon with energy $h\nu$. Characteristic radiation and Auger electrons are emitted as electrons cascade to replace the ejected photoelectron.

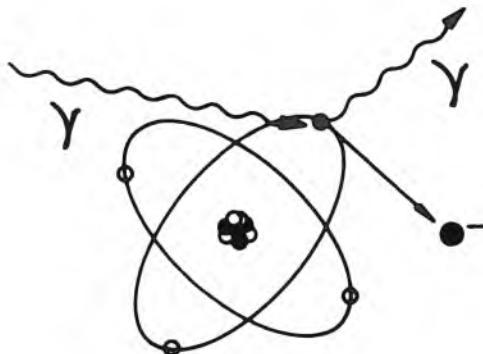
electric absorption is dominant up to a photon energy of 50 keV. A diagrammatic representation of the photoelectric effect is shown in Figure 3.10.

B. THE COMPTON EFFECT

An incident photon of energy ($h\nu$) collides with the electron to produce a recoil electron at a given angle and a scattered photon with reduced energy $h\nu$. This process is called the Compton effect. The Compton process involves an interaction between a photon and a "free," or unbound, electron. It is independent of atomic number and decreases with increasing energy. A diagrammatic representation of the Compton effect is shown in Figure 3.11.

C. PAIR PRODUCTION

In this process, the photon passes near the nucleus of the atom and is subjected to the strong field of the nucleus. During this event, the photon suddenly disappears and becomes a positive



$$\text{Energy of recoil electron} = \\ \text{energy of photon} - \\ (\text{B.E. of electron} + \text{energy} \\ \text{of scattered photon})$$

FIGURE 3.11. Schematic representation of the Compton effect.

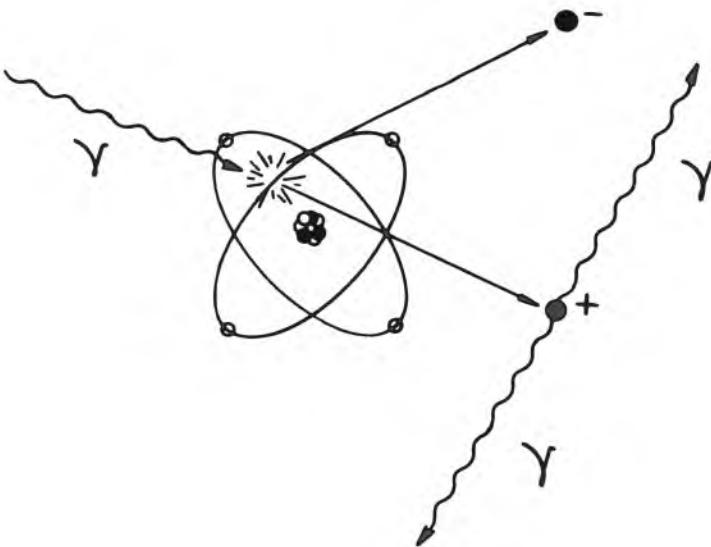


FIGURE 3.12. Schematic representation of pair production.

and negative electron pair (Figure 3.12). This is a good example of conversion of energy into mass. The process must be considered as a collision between the photon and the nucleus; in this collision, the nucleus recoils with some momentum. It also attains a little energy, but the energy involved is too small in comparison with the energies given to the positron and electron and, therefore, can be neglected. Because the energy equivalent to one electron mass is 0.511 MeV, and because two particles are formed, the minimum energy of the incident photon to produce pair production must be 2×0.511 or 1.022 MeV.

IX. SUMMARY OF ABSORPTION OF RADIATION IN SOFT TISSUE

A. PHOTON ENERGY

1. Up to 50 keV: photoelectric absorption is important
2. 60 to 90 keV: photoelectric and Compton effects are equally important
3. 200 keV to 2 MeV: Compton absorption is dominant
4. 5 to 10 MeV: pair production begins to be important
5. 50 to 100 MeV: pair production is the most important type of absorption

B. COHERENT SCATTERING

Photons are scattered with negligible loss of energy during coherent scattering.

C. PHOTODISINTEGRATION

Photodisintegration occurs with a threshold X- or γ -ray energy of 1.65 MeV. A beryllium foil emits neutrons after irradiation by photons of energy in excess of 1.65 MeV. For example:

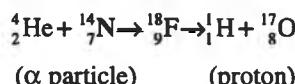


A silver foil adjacent to the beryllium is activated by the neutrons and emits γ -rays.

X. TYPES OF ACCELERATORS

A. BACKGROUND OF ACCELERATOR DEVELOPMENT

Rutherford produced nuclear disintegration for the first time by bombarding the low-atomic-number elements with α particles obtained from radioactive substances. No nuclear disintegration was induced in high-atomic-number elements by this technique. Rutherford and Chadwick bombarded all the light elements with α particles and found about ten cases where protons were produced. By studying the range of these protons, they determined that in some cases the protons had more energy than the original α particles. This showed that the α particles were absorbed by the nucleus, which then ejected protons whose energy was largely dependent on the instability of the intermediate nucleus. For example:



B. THE CROCKROFT-WALTON EXPERIMENT

The first nuclear disintegration induced without the help of α particles from radioactive substances was made by Crockroft and Walton in the same year (1932) that Chadwick discovered the neutron. They used protons as bombarding particles. The protons were accelerated by a high voltage, which was achieved by charging capacitors in parallel and discharging them in series — a technique in use at that time in X-ray technology. Proton energy up to 0.7 MeV was obtained in this way. It was already known from Rutherford's experiment that alpha bombardment often produced protons, but they also found that proton bombardment often produced α particles. For example:



C. VAN DE GRAAFF ELECTROSTATIC GENERATOR

The particle accelerator, or "atomic smasher," consists of an ion source, an evacuated region in which ions can be accelerated, and a source of high potential to do the acceleration. The only high-voltage system in wide use today is the Van de Graaff electrostatic generator, in which charges are transported to bodies by means of a moving belt to supply the required large potentials. Accelerators of this type can supply particles of up to a few million electron volts.

D. CYCLOTRON

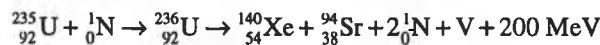
In 1932 Lawrence invented the cyclotron, which made it possible to attain higher-energy particles by using a comparable low voltage over and over. Charged particles moving with a velocity perpendicular to a magnetic field move in a circular path. Lawrence used this principle in designing the cyclotron. The main features of the cyclotron consist of two chambers called D s (because of the shape). The ions to be accelerated are introduced into the center of these D s. If D_2 is negative relative to D_1 , the positive ion is attracted into D_2 where it experiences no electrical field because it is within a conducting chamber. A magnetic field, perpendicular to the plane of the D s, forces ions into a path with angular velocity. In time, the ion returns to the gap between the D s; if at this time the potentials at the D s are reversed, the ions are again accelerated in going from D_2 into D_1 . This process is repeated again and again. Large cyclotrons can produce 10-MeV protons, 20-MeV deuterons, and 40-MeV α particles. The real upper limit of cyclotrons is set by the relativistic mass variation of the ions

accelerated. As the velocity of the ions approaches the velocity of light, the mass increases and the angular velocity is no longer constant. Since 1932, a tremendous development in accelerator technology has occurred. The largest machine, located in Illinois, produces particles of 200 BeV. Some of the accelerators that are being used are listed below.

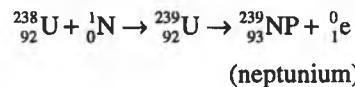
1. The **synchroncyclotron** provides proton energy up to 350 MeV.
2. The **proton synchrotron** the first proton synchrotron in the multi-BeV range was the *Cosmotron* at Brookhaven National Laboratory, Upton, NY. It provides proton energy up to 3 BeV.
3. The **betatron** uses electrons instead of protons. Energy of 100 MeV is readily obtained in a betatron.
4. The **electron synchrotron** provides electron energy up to about 1 BeV.

E. PRINCIPLE OF THE NUCLEAR REACTOR

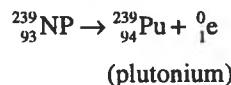
The principal isotope of uranium (U) that undergoes fission is ^{235}U . The fission reaction for ^{235}U can be written as follows:



Both ^{140}Xe and ^{94}Sr are radioactive β emitters. In addition to the fission of ^{235}U , two more reactions were found:



and



Neptunium has a half-life of 2.33 days, whereas plutonium has a half-life of 24,400 years and is a fissionable element. Natural uranium contains 99.3% ^{238}U and only 0.7% ^{235}U . The natural uranium cannot sustain a chain reaction for two reasons: (1) neutrons from the first fission might escape from the sample, and (2) neutrons might get captured by nonfissionable elements such as ^{238}U . In 1942, many physicists believed that a chain reaction would occur in pure ^{235}U and possibly in an ordinary uranium sample in a properly moderated reactor. The criteria for the moderator were that the elements must be of low atomic material to provide an inelastic collision and at the same time have a very low cross section for neutron capture. Deuterium and carbon are commonly used for this purpose.

The first experimental reactor was assembled by Fermi and associates at the University of Chicago. It was a cubic lattice of lumps of uranium in a *pile* of graphite blocks. The lump size was a compromise (10 cm in diameter). They were made large in order to include as many ^{235}U nuclei as possible, and yet small enough so that most of the fast-fission neutrons escaped without being captured by ^{235}U . The graphite space between the lumps was chosen so that most of the neutrons from one lump would be thermalized by the time they reached the next lump, about 30 cm away. The reactor was built around a neutron source using α particles from a radioactive source to excite an (α, n) reaction, as in beryllium. The pile first became critical on the afternoon of December 2, 1942, and a chain reaction was finally achieved.

XI. DOSIMETRIC CONSIDERATION

A. MEASUREMENTS OF RADIATION

To have a good understanding of radiation injury, the precise measurement of the radiation quantity delivered to a biological system is essential. In 1962, the International Commission on Radiological Units and Measurements defined the unit of quantity of ionizing radiation more explicitly. Exposure of X- or γ -radiation is a measure of radiation in air and is based upon the ionization produced. The roentgen (R) is a unit of exposure. An exposure of 1 R measures electric charges of either sign produced in 0.001293 g or 1 cm³ of air at standard temperature and pressure. R measured by the ionization chamber should be corrected for the temperature and pressure by the following formula:

$$\frac{t+273}{273} \times \frac{760}{P}$$

where t is the room temperature in degrees Celsius and P is room atmospheric pressure in millimeters. This correction factor can also be obtained from a table supplied by General Electric, Inc. The R is applicable to X- and γ -radiation below 3 MeV. Therefore, this radiation unit should not be used with a charged-particle beam or with X-ray and γ -photons above 3 MeV. The Victoreen ionization chamber is commonly used for measuring the exposure rate. This chamber and the R value on the roentgen scale should be calibrated before use. In addition, the R value on the roentgen scale should be read between 20 and 80% value of the scale. During radiation measurement, the elements of high atomic number must be removed from the radiation field, because interaction of the primary beam with this material produces scattered radiation of low energy. The devices in which biological materials are irradiated are usually elements of low atomic number. Plastic is most commonly used for this purpose.

The X-ray machine (250 kVp) produces a heterogeneous beam of radiation with energy varying from a few electron volts up to 250 keV, the average energy being one-third of kVp. Therefore, a proper filter must be used in order to obtain a relatively homogeneous X-ray beam. The combination of 0.5 mm Cu and 1 mm Al is suitable for this purpose. Copper absorbs soft X-rays, whereas aluminum absorbs the characteristic X-rays of copper.

The quality of the X-ray beam coming through the filter should be stated in terms of half-value thickness (hvt), which refers to the thickness of the absorbing material (Cu is most commonly used) that is needed to reduce the initial radiation intensity by one-half. Many investigators prefer to express the quality of the beam in terms of the homogeneity coefficient, which is the ratio of the first hvt over the second hvt.

Dose: This is a measure of radiation which is based upon the absorption of 100 erg of energy per gram of any substance. The unit of dose is the *rad*. Roentgens can be converted to rads by the following formula: $f \times R = \text{rad}$, where f is a conversion factor — the value of which is based on the hvt of the beam. These values are listed in Table 3.1.

Gy: To honor the contribution of Dr. Gray, a new unit, the Gy, was proposed to measure radiation dose: 1 Gy = 100 rads.

Nominal standard dose (NSD):

$$\text{NSD} = \frac{D}{N^{0.24}} \times t^{0.11}$$

where D = rads, N = number of fractions, and t = time of therapy in days. NSD provides an opportunity to compare results of two different radiation treatment centers using different radiation therapy modalities. NSD is expressed as radiation equivalent therapy (ret).

$$\text{Rem} = \text{Rad} \times \text{RBE}$$

$$100 \text{ Rem} = 1 \text{ Sievert (Sv)}$$

B. MEASUREMENT OF R FROM GAMMA-EMITTING RADIOISOTOPES

It is possible to estimate the radiation exposure for γ -rays with energy between 0.3 and 3.0 MeV by the following equation: $R = 6 \text{ CiE}$, where R is the exposure rate (R/h at 1 ft), Ci is the number of curies, and E is the average γ energy per disintegration (MeV). If γ energies greater than one are emitted per disintegration, this should be taken into consideration. For example, ^{60}Co has a γ energy of 1.2 and 1.3 MeV; therefore, the E value for ^{60}Co would be $1.2 + 1.3 = 2.5$ MeV. Another relationship that can be used with the γ source is the inverse square law, which states that the radiation intensity varies inversely as the square of the distance from the source.

The measure of the radiation quality of a radioisotope that is located inside the body and that emits β - or γ -rays is more complicated. Because of the short range of these particles in tissue (usually 1 cm or less), it is often assumed that most of the energy is absorbed within the particular organ. The general formula for dose rate is:

$$\tilde{D} = \tilde{\text{Dose}} \text{ (rad/day)} = \frac{A(\mu\text{Ci}) \times 2.2 \times 10^6 \text{ (dpm}/\mu\text{Ci}) \times 1440 \text{ (min/day)} \times \bar{E} \text{ (MeV)}}{W(\text{g}) \times 6.24 \times 10^5 \text{ (MeV/erg)} \times 100 \text{ (erg/g/rad)}}$$

where A is the total radioactivity in the system, W is the weight of the system in which A is distributed, and D is the average dose rate in the system. This factor does not take into account any nonuniform distribution. E , the average energy per disintegration, equals the total energy for an α -emitter or a third of the maximum energy for a β -emitter.

The total dose, which is absorbed over a period of time, can then be calculated from the initial dose rate if the effective half-life of the radionuclide is known. The dose accumulated up to a given time (t) after the introduction of the nuclide is:

$$\text{Dose (rad)} = 1.44 \times T_e \text{ (days)} \times D \text{ (rad/day)} \times \left(1 - \frac{\bar{E}}{T_e} \times \frac{0.693t}{T_e} \right)$$

where T_e represents the effective half-life of radionuclides. If the time t is very long relative to the effective half-life, the total dose is:

TABLE 3.1
Conversion Factor f as a Function
of Energy and Type of Tissue*

Energy (MeV)	Conversion Factor (f)		
	Water	Bone	Muscle
0.01	0.920	3.58	0.933
0.10	0.957	1.47	0.957
1.0	0.974	0.927	0.965

* Data from International Commission on Radiological Units and Measurements, Handbook 62, National Bureau of Standards, Washington, D.C., 1965.

$$\text{Rad} = 1.44 \times T_e \text{ (days)} \times D \text{ (rad/day)}$$

The effective half-life includes both the physical half-life and the biological half-life (time to reduce the radioactivity in the body or in the organ to one half)

$$T_b = \frac{T_p \times T_e}{T_p - T_e}$$

where T_p is the physical half-life and T_b is the biological half-life.

C. LUMINESCENT DOSIMETERS

Several types of luminescent dosimeters are available, and a very wide range of doses can be measured by using the thermoluminescent phenomenon. Lithium fluoride, which shows this effect, has an atomic number close to that of soft tissues; therefore, the absorption coefficient in both types of materials is similar. Because only about 10 mg of lithium fluoride powder is needed for measurements, the method is very suitable for measuring the dose during radiation therapy. The tiny lithium fluoride capsules are readily inserted into body cavities and into sites that are not normally accessible to a more conventional device. The thermoluminescent phenomenon is based on the fact that when lithium fluoride is exposed to ionizing radiation, electrons are excited into higher energy levels. They then fall into "electron traps," leading to a metastable state. When the energy is applied in the form of heat, the trapped electrons return to the ground state with an emission of light. The radiation dose can be determined by measuring the intensity of emitted light.

D. CHEMICAL DOSIMETERS

The ferrous-ferric system, often called the *Fricke dosimeter*, is also used. A well-oxygenated solution of pure ferrous ammonium sulfate (10^{-3} M) in diluted sulfuric acid (0.8 M) is exposed to radiation. Ferrous ions are oxidized to ferric ions in an amount dependent on the total amount of energy deposited in the system. The quantity of ferric ions produced is then determined in a UV spectrophotometer. The most suitable range for this dosimeter is between 5000 (50 Gy) and 50,000 rad (500 Gy), which makes the Fricke dosimeter of limited value in the dose range of biological interest.

E. PHOTOGRAPHIC FILM

Photographic film was one of the first radiation detectors and is still widely used for personnel monitoring. The photographic film is calibrated in terms of the gross blackening of the film after radiation exposure. The intensity of darkness of the film, which is measured by a densimeter, is proportional to the energy of the photon. This is suitable within a very low exposure range.

XII. SUMMARY AND COMMENTS

We have available more accurate dosimeters, thus we can establish the dose-effect relationship more precisely. Several types of high-energy machines are available for producing high LET radiation at high dose rates. These types of radiation can be used to study the biological effect of high LET radiations. The introduction of Gy instead of rad as a unit of radiation dose has created unnecessary confusion in the literature.

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Chapter 4

CELLULAR RADIATION DAMAGE

CONTENTS

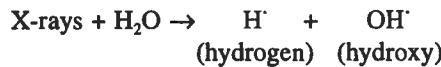
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I. MODES OF RADIATION INJURY

When cells are irradiated, damage is produced primarily by *ionization* and *free radicals*.¹⁻³ Low LET radiations such as X- and γ -rays produce damage primarily by free radicals, whereas high LET radiations such as protons and α particles produce damage primarily by ionization. Ionized molecules are inactive. The energy released per ionization is about 33 eV, which is sufficient to break a chemical bond. For example, the energy associated with a C=C bond is only about 4.9 eV.

A. FREE RADICALS

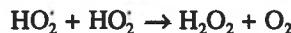
Free radicals are neutral atoms or molecules having an unpaired electron. When X-rays interact with water, two types of free radicals are formed:



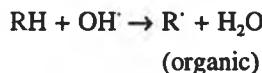
The recombination of free radicals yields the following:



The presence of an excess of oxygen during irradiation of cells allows the formation of additional free radicals:



When organic molecules (RH) combine with hydroxy free radicals, the organic free radical (R') is formed.



The organic free radicals (R') then combine with O₂ to form peroxy (RO₂[·]) free radicals:



Thus, the presence of excess oxygen allows the formation of two additional free radicals, hydroperoxy (HO₂[·]) and peroxy (RO₂[·]). This may, in part, account for the increased radiation damage in the presence of excess of oxygen.

Lifetime of free radicals: Most of the free radicals are very short-lived and readily combine with each other. The lifetime of free radicals is generally less than 10⁻¹⁰ sec. However, there are a few free radicals derived from complex organic substances that are stable and do not readily combine.

Measurement of free radicals: The amounts of free radicals produced by irradiation can be measured by electron spin resonance, which measures the unpaired electron.

Reactivity of free radicals: Because free radicals contain unpaired electrons, they are very reactive and can oxidize or reduce the biological molecules within the cell. The free radicals OH[·] and HO₂[·] are oxidizing agents, whereas H[·] is a reducing agent. Free radicals can damage molecules such as DNA, RNA, and proteins as well as membranes. Free radicals have been implicated in the etiology of cancer as well as in neurodegenerative diseases.

B. EFFECT OF LET AND HIGH DOSE RATE ON IRRADIATED WATER

The irradiation of aerated water with a high total dose of low LET radiation (X- or γ -ray) produces an appreciable amount of H₂O₂; however, if the water is oxygen-free, no detectable amount of H₂O₂ is formed. But irradiation of oxygen-free water with high LET radiation (α particles, protons, and neutrons) after a similar total dose, produces detectable amounts of H₂O₂. The dose rate effect of low LET radiation on cell survival is observed only at a very high dose rate and is equivalent to high LET radiation effect.

C. ESTIMATION OF DAMAGE PRODUCED BY IONIZATION AND FREE RADICALS

Radiation injuries are produced by both ionization and free radicals. How much one type of effect in a given biological system contributes to the total damage depends upon experimental conditions. There is no assay technique that can quantify the damage produced by

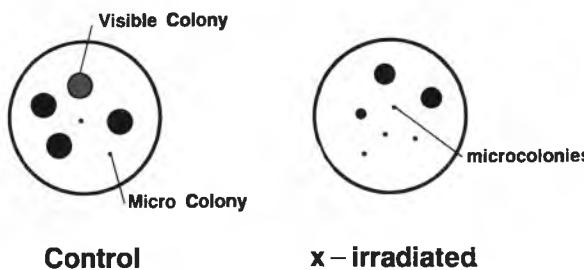


FIGURE 4.1. Diagrammatic representation of formation of colonies in control and irradiated tissue culture dishes. Note the increased number of microcolonies in irradiated cells in comparison to control cells.

ionization and free radicals separately; however, one can perform a series of experiments that might help in estimating whether a given damage is primarily due to ionization or free radicals. Those radiation effects that are modifiable by preexposure treatment with chemicals may be primarily due to indirect action. The damage produced by ionization is not modifiable by the above treatment. It has been estimated that about two-thirds of biological damage by low LET radiation is due to indirect action. Biological damage by high LET is primarily by ionization.

D. SURVIVAL CRITERIA

An *in vitro* survival criterion of mammalian cells generally is based on the ability of individual cells to divide and form recognizable *colonies* in a plastic dish (Figure 4.1). The plating efficiency (PE) refers to the percentage of cells that are able to form visible colonies. A population of cells derived from a single parent is referred to as a *clone*, whereas the population of cells derived from more than one parent is called a *colony*. Depending upon the growth properties of the cells being used, and upon possible alterations of these properties by irradiation, the time required for the appearance of a recognizable colony under optimal growth conditions may vary. Generally, the time interval between plating and colony formation is about 10–14 days. For the correct interpretation of the survival curve, it is desirable to have a cell line that has 100% plating efficiency. However, the trauma associated with suspension, dilution, and plating can lead to lower PE.

Although colony formation technique is commonly used to determine viability of cells in radiobiological studies with cell culture, it has the following limitations:

1. The visible colonies are often associated with microcolonies, many of which are primarily the result of slow growth rate rather than reproductive death.
2. The distinction between visible colonies and microcolonies is subjective; therefore, many microcolonies that are ignored could be the result of slow growth rate rather than reproductive death. The issue of microcolonies becomes crucial in irradiated culture where many such colonies are formed.

The growth inhibition, which is the result of cell death and slow growth rate, is often criticized because it does not measure reproductive death precisely. However, the colony formation technique overestimates the radiation injuries, because the microcolonies are arbitrarily not counted. Therefore, the growth inhibition is a good measure of radiation injury.

E. TYPES OF CELL SURVIVAL CURVES

The two most common types of survival curves obtained after irradiation of mammalian cells *in vitro* are the exponential (Figure 4.2A) and sigmoid curves (Figure 4.2B). These are similar to single-hit or single-target and multi-hit or multi-target curves of target theory. Since

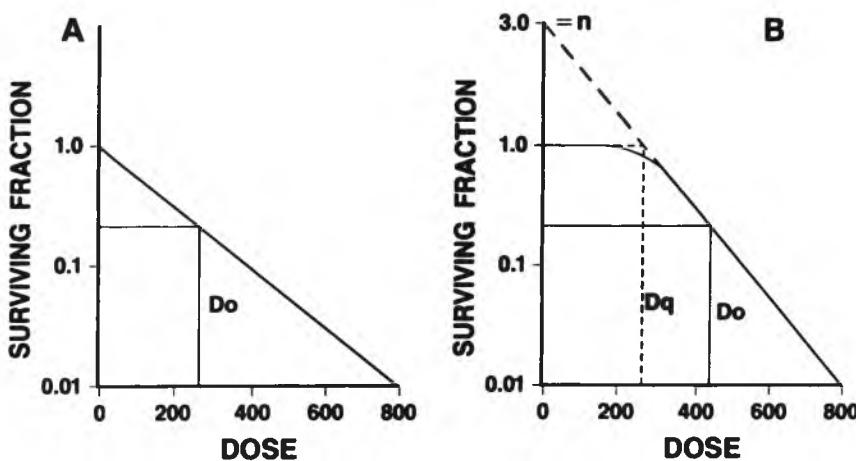


FIGURE 4.2. Diagrammatic representation of mammalian cell survival curve, showing exponential form (A) and sigmoid form (B).

we do not know whether the mechanisms of radiation-induced cell killing involve one-hit or one-target, multi-hit, or multi-target, the target theory of radiation effect may not be fully applicable to explain the mechanisms of radiation damage. The surviving fraction (log) is often plotted as a function of radiation dose (linear).

F. PARAMETERS OF SURVIVAL CURVES

Each survival curve after irradiation is characterized by the following parameters:

PE = Plating efficiency. Percentage of cells able to form large colonies.

D_q = The quasithreshold dose for a given cell population. The value of D_q depends upon experimental conditions and cell type. It often measures width of shoulder and reflects approximately a threshold dose for a given effect.

D₀ = The dose that reduces the surviving fraction to 1/e (= 0.37) on the exponential portion of the curve, or the dose that produces 37% survival. The value of D₀ also depends upon the experimental conditions and cell type. D₀ for most mammalian cancer cells is about 200 rads (2 Gy), and for humans it is about 300 rads (3 Gy).

n = Extrapolation number. This value is obtained by extrapolating the exponential portion of the curve to the abscissa. The value of n also depends upon the experimental conditions and cell type. It also measures the width of shoulder.

N = Cellular multiplicity. The number of cells per colony at the time of exposure.

$$\text{Surviving Fraction} = \frac{\text{Colonies counted}}{\text{Cells plated} \times (\text{PE}/100)}$$

G. INTERPRETATION OF SURVIVAL CURVES

Alper⁴ has made an excellent review of various models used in interpretation of radiation survival curves.

1. Linear Hypothesis

The linear hypothesis for radiation lethality to human cells at low doses was tested.⁵ It was found that low doses (21–87 rads) of X-rays inactivated the colony-forming ability of irradiated human kidney cells (T-1) in culture. At a dose of 21 rads, the split dose technique

failed to reveal any repair of sublethal damage.⁵ Therefore, it was concluded⁵ that the linear hypothesis is a valid modality for estimating the inactivation of the human cell at low doses. This hypothesis states that the decrease in surviving function is proportional to the dose.

The exponential curve obtained after irradiation is not necessarily the result of a single-hit action or a single sensitive target, but rather a sum of several factors. This is particularly true when the exponential curve is modifiable by exogenous agents.

There are two interpretations of the shoulder obtained after irradiation of mammalian survival curve:

1. The shoulder region shows the extent of accumulation of sublethal damage before cells lose reproductive capacity.³
2. The shoulder region shows a repair process that operates at the outset of irradiation, but it becomes less and less effective as the dose increases, until the processes of damage continue without concomitant repair.^{6,7}

Sinclair has suggested⁸ that there is a factor, referred to as Q, which may regulate the magnitude of the shoulder of the survival curve as cells proceed through the cell cycle. Alper has separated the Q factor into Q-repair and Q-lesion.⁹ Thus, it has been proposed⁹ that the existence of a shoulder in the survival curve is due to the presence of Q-repair. If the cells are defective in Q-repair, they will be killed exponentially; or, if the radiation doses are high enough to destroy Q-repair, cells will be killed exponentially because of the dominance of Q-lesion.

2. Quadratic Hypothesis

The surviving function decreases as a function of the square of the dose.

3. Linear-Quadratic Hypothesis

This model suggests that there are two components of cell killing. One is proportional to the dose (linear), and one is proportional to the square of the dose (nonlinear). These two components of the curve may result from the same cellular damages that are progressing at a different rate, and not from two independent processes of damage.

II. CONCEPT OF RADIOSENSITIVITY

In comparing the radiosensitivity of different types of cells, tissues, or organs, one must define the criterion of radiation damage. A statement about the radiosensitivity of cells without reference to the criterion of radiation damage is meaningless. Some cells may be radioresistant as judged by one criterion of damage, but highly radiosensitive by another. A few principles that allow one to predict the radiosensitivity of cells are described in this chapter. Some major references are listed at the end of the chapter.^{3,10}

A. LAW OF BERGONIÉ AND TRIBONDÉAU

In 1906, Bergonié and Tribondéau,¹¹ while working on the effects of radiation on the rat testis, formulated a concept of the radiosensitivity of cells that has been held to be remarkably true in its general terms. The law of Bergonié and Tribondéau states:

X-rays are more effective on cells that have a greater reproductive activity; the effectiveness is greater on those cells that have a longer dividing future ahead — on those cells of which the morphology and the function are the least fixed, thus in direct

proportion to their reproductive activity and inversely proportional to their degree of differentiation.

Indeed, using the criterion of cell death, rapidly dividing systems (bone marrow, gonad, and intestine) are more radiosensitive than the nondividing systems (liver, kidney, brain, etc.). Furthermore, the undifferentiated cell types are more sensitive to X-irradiation than the differentiated ones. The peripheral lymphocytes and the oocytes of mammals represent an exception to the law of Bergonié and Tribondéau. Both lymphocytes and oocytes are highly radiosensitive even though they are neither dividing nor differentiating. We do not know why the lymphocytes and oocytes are so radiosensitive. It has been reported¹² that the level of adenosine 3',5'-cyclic-monophosphate (cyclic AMP) may be inversely related to the radiosensitivity of cells. Because rapidly dividing cells have a lower level of a cyclic AMP than those that are slowly dividing, cyclic AMP may provide a molecular basis for the law of Bergonié and Tribondéau. The law of Bergonié and Tribondéau is not always applicable to tumor cells *in vivo*, especially tumor cells that have regrown after the completion of first radiation therapy.

B. RELATIONSHIP BETWEEN RADIOSENSITIVITY AND INTERPHASE CHROMOSOME VOLUME

Sparrow¹³ has proposed that the radiosensitivity of cells is directly proportional to the interphase chromosomal volume, the criterion of radiosensitivity being the cell death or growth inhibition (Figure 4.3). Because the DNA content increases with increasing nuclear or chromosomal volume, a direct relationship also exists between the estimated amount of DNA per chromosome and the radiosensitivity of cells. In defense of his hypothesis, Sparrow has argued that one must consider not only uniformity in the physical dosimetry, but also in the biological dosimetry. Therefore, to compare the response of two types of cells one must express the biological dosimetry in terms of the energy absorption per chromosome. However,

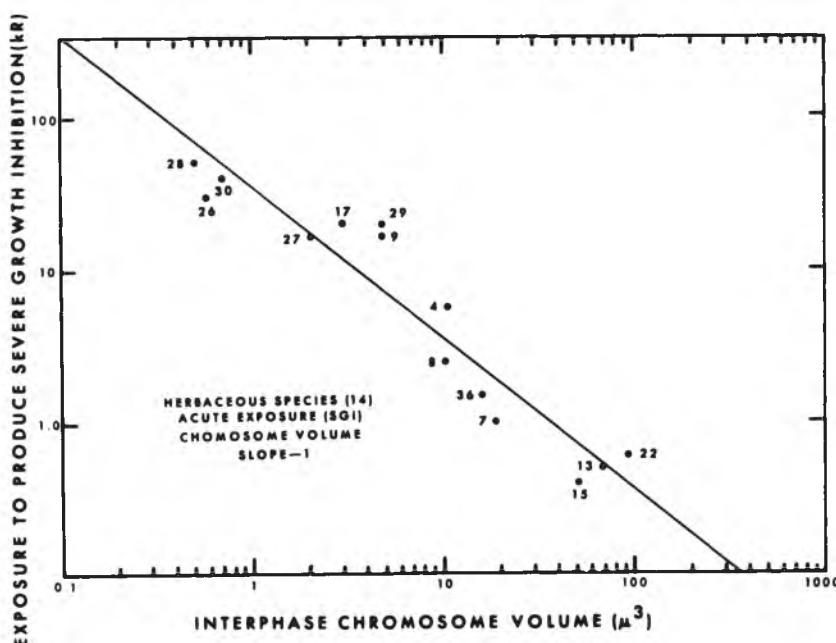


FIGURE 4.3. Regression of exposure in kiloroentgens required to produce severe growth inhibition against interphase chromosome volume for 14 herbaceous species following acute irradiation, plotted with slope of -1. (From Sparrow, A.H., *Cellular Radiation Biology*, Williams & Wilkins, Baltimore, 1965, 207.)

in most radiobiological studies, energy absorption per unit volume or weight of air or tissues has been measured.

It is well established that the chromosome number and volume differ from one species to another in both plants and animals. Therefore, energy absorption per chromosome would vary markedly from one species to another in spite of uniformity in the physical dosimetry. This may account for the wide variation in the radiation response of different species. Therefore, Sparrow has suggested that if one expresses the radiation dose as energy absorption per chromosome, the apparent difference in the radiosensitivity among various species may largely disappear. This situation is especially true when one compares the radiation responses of polyploid cells with those of diploid ones. For a long time it had been presumed that the greater resistance of polyploid cells to irradiation was due to their genetic redundancy, but this may not be true. Polyploid cells have less interphase chromosome volume than that of diploid ones. This may account for their radioresistance. All plant cells thus far examined have shown a direct relationship between radiosensitivity and interphase chromosomal volume. The applicability of this principle for animal cells has not been adequately investigated. It is essential that one considers the uniformity of the biological dosimetry as well as the relative kinetics of cell growth while comparing the radiosensitivity of two cell types.

C. ROLE OF CYTOPLASMIC STRUCTURES IN RADIOSENSITIVITY

Although the nucleus is considered to be the most radiosensitive spot in the cells for many types of cellular and molecular damage, Goldfeder¹⁴ has proposed that, for certain cell types, mitochondria may be an important factor in determining radiosensitivity. She has demonstrated that epithelial tumors, which have an abundance of mitochondria, are more radioresistant than the spindle-cell tumors, which have fewer mitochondria. The radiosensitivity was determined by measuring the regression of tumors following irradiation *in situ*. To further justify her hypothesis, she compares the radiation response of the lymphocytes and the heart muscle cells. The former cell type has few mitochondria but is very sensitive to irradiation, whereas the latter cell type has an abundance of mitochondria but is very radioresistant. The assumption for the above hypothesis is simple. If the cells have many mitochondria, damage of an appreciable fraction of them may not be lethal for cells. On the other hand, if the cells have only a few mitochondria, damage of even a few of them may have severe consequences on cell function. There appears to be a good correlation between the number of mitochondria and radiosensitivity of some cell types. Whether there is a cause-and-effect relationship is uncertain.

D. RADIOSENSITIVITY IN RELATION TO THE CELL CYCLE

The identification of four phases in a cell cycle, mitosis (M), pre-DNA synthesis (G_1), DNA synthesis (S), and post-DNA synthesis (G_2), and success in the synchronization of cells, have increased our understanding of cellular radiosensitivity. The following principles on the radiation response of a synchronized cell population have been established: (1) on the basis of the same criterion of radiation damage, the radiosensitivity of cells varies as they move from one phase of the cell cycle to another; (2) for the same criterion of radiation damage, the sensitivity of a given phase of the cell cycle differs from one cell type to another; and (3) the sensitivity of a given phase of the cell cycle changes if the criterion of radiation injury is altered. Some examples are given in Table 4.1.

E. RELATIVE RADIOSENSITIVITY OF CELLS DURING GROWTH

When monolayer cultures of mammalian cells are allowed to reach the stationary or plateau phase of growth, overall DNA synthesis markedly decreases. This is chiefly a result of the appearance of many nonproliferating cells that remain in the pre-DNA synthetic (G_1) phase of the cell cycle, while the cell concentration remains approximately constant because the lowered rate of cell division is balanced by sloughing off the dead cells into the nutrient

TABLE 4.1
Radiation Sensitivity of Various
Phases of the Cell Cycle

Criteria of Radiosensitivity	Most Sensitive Phase of Cell Cycle
Reproductive death	M
Chromosomal damage	G ₂
Division delay	G ₂
DNA synthesis	Early G ₁

medium. Stationary-phase cultures of mammalian cells are an interesting *in vitro* cell system, for they exhibit certain characteristics of more complex *in vivo* systems such as those in tumors. It is well known that human tumors contain a sizable fraction of nonproliferating but potentially colony-forming cells. The modification of radiation damage in this particular fraction of cells is important, because the effectiveness of radiation therapy would depend upon how effectively these cells are killed by ionizing radiation.

When Chinese hamster cells were irradiated in the plateau phase of growth, the slope of the survival curve was similar to that obtained with exponentially grown cultures, but the cells did not accumulate and repair sublethal damage.⁶ D₀ values for cells growing in the exponential and stationary phases were 142.3 ± 4.3 and 136.4 ± 0.68 rads (cGy), respectively. It has been shown that normal human liver cells, when irradiated in the plateau phase of growth, repair sublethal damage. These cells also repair potentially lethal damage if the cells are allowed to remain in the stationary phase for some time after irradiation. The slopes of the survival curves differed significantly; the D₀ was consistently 20–30 rads lower with stationary phase than with exponentially grown cultures, while the extrapolation number was slightly greater. Thus, the relative radiation response of cells growing in the exponential and stationary phases seems to be influenced by cell types, specific culture conditions, and criteria of radiation damage.

F. TYPES OF CELLULAR INJURY

It is well established that the extent of radiation damage of a given cell type depends upon the total dose, dose rate, type of radiation (high LET vs. low LET), mode of radiation delivery (single dose vs. fractionated dose), and the environmental condition of the medium. The types of radiation injuries in relation to the approximate doses are given in Table 4.2.

G. RADIOSENSITIVITY OF NUCLEUS VS. CYTOPLASM

The basis for the study of the effects of X-irradiation on the cytoplasmic level is a simple one. The metabolic processes of the cells are well-coordinated by the nuclear and cytoplasmic

TABLE 4.2
Types of Cellular Damage in Relation to Approximate Dose

Dose (rads)	Type of Damage	Comments
1–5	Mutation (chromosomal aberration, gene damage)	Irreversible chromosome breaks, may repair
100	Mitotic delay, impaired cell function	Reversible
300	Permanent mitotic inhibition, impaired cell function, activation and deactivation of cellular genes, and oncogenes	Certain functions may repair; one or more divisions may occur
>400–1000	Interphase death	No division
50,000	Instant death	Proteins coagulate

TABLE 4.3
Radiosensitivity of Nucleus vs. Cytoplasm

Cell Type	Area Irradiated	Dose	Mortality (%)
Egg of insect (<i>Habrobracon</i>)	Nucleus	1 α particle	100
	Cytoplasm	16 million α particles	50
Amoeba	Nucleus	150 kR	50
	Cytoplasm	290 kR	50
Newt heart cells	Nucleus	A few protons	High
	Cytoplasm	Several thousand protons	None
Chinese hamster fibroblasts	Cytoplasm	725,000 rads (α particles)	None
	Nucleus	26 rads	50

organelles; therefore, if radiation causes any abnormality in cell function, it may be reflected in the structural changes of the cytoplasmic organelles, and vice versa.

The electron microscope has served as a powerful tool in visualizing the morphological changes of the cellular organelles. The extent of radiation damage to the cellular organelles depends upon radiation factors such as total dose, dose rate, mode of radiation delivery (single dose vs. fractionated dose), type of radiation (high LET vs. low LET), and so on. The cytoplasmic changes after irradiation include swelling, vacuolization, and disintegration of mitochondria and endoplasmic reticulum. Changes in the Golgi apparatus and polysomes are difficult to see; however, if cells have short-lived messenger RNA, one may observe a reduction in the number of polysomes. The nuclear changes include swelling of nuclear membrane and disruption of chromatin materials. It should be emphasized that the morphological changes seen after irradiation are very nonspecific, because a fixative artifact may induce structural alterations similar to those caused by X-irradiation. Therefore, considerable caution must be exercised in interpreting electron microscopic observations after X-irradiation.

In general, the nucleus is considered relatively more radiosensitive than the cytoplasm on the criterion of reproductive death. Table 4.3 summarizes the evidence of nuclear vs. cytoplasmic radiosensitivity.

H. RADIATION EFFECT ON MITOSIS AND MEIOSIS

On the criterion of cell death, mammalian cells are most radiosensitive during the M period of the cell cycle on the criterion of reproductive death. Depending upon the radiation dose, mitotic cells may or may not show chromosomal damage. The damage to chromosomes may lead to mutation. It has been suggested that X-ray-induced cell death results from damage to chromatin structures.¹⁷

The effect of radiation on the process of meiosis is poorly understood and needs further investigation. This process of cell division in mammals is observed only during spermatogenesis or oogenesis. It has been shown that during spermatogenesis, when meiosis is altered by radiation, the division of primary and secondary spermatocytes is so affected that cells with double nuclei or fused giant nuclei appear.

The radiation response of males differs greatly from that of females and can be correlated with the striking difference in gametogenesis, because spermatogonia continually form mature spermatocytes, whereas in the female the definitive supply of oocytes is formed approximately at the time of birth. Therefore, radiation damage in females is accumulative, whereas it is variable in males, because many damaged cells can be eliminated in males.

I. DIVISION DELAY

The division delay, after irradiation, increases with dose. The division delay also varies from one cell type to another and during the various phases of the life cycle. For mouse L cells, division delay is 2–3 hr/100 rads (cGy) for HeLa cells, division delay is about 1.6 hr/100 rads (cGy). HeLa cells midway in the G₁ phase have a relatively short division delay per unit dose, and that division delay increases as cells progress through the S and G₂ phases.

J. EFFECT OF IRRADIATION ON THE MOVEMENT OF CELLS THROUGH THE CELL CYCLE

Slowly and rapidly dividing cells show differing radiosensitivity in relation to the movement of the cell through the cell cycle.¹⁸

Progression of cells from G₁ phase to S phase: In human amnion cells (generation time 14 to 20 days, most cells in the G₁ phase) there was a dose-dependent decrease in the rate of flow of G₁ cells into S with 10–100 rads (cGy) and the progression was almost completely stopped for 3–10 days by 300–1000 rads (cGy). No such effect was observed with the rapidly proliferating cells.

Progression of cells through S phase: An exposure of 1000 rads (cGy) reduced the progression of cells already in the S phase by about 30%.

Progression of cells from S phase to G₂ phase: The progression of cells from the S to the G₂ phase was not significantly affected by exposure to 100–1000 rads (cGy). This was similar to that in the rapidly dividing cells.

Progression of cells from G₂ phase through M phase: Exposure to 100–1000 rads (cGy) induced delay in the passage of cells from the G₂ phase through the M phase. The extent of delay was dose-dependent and also proportional to the long generation time. The G₂ block was reversible. When a rapidly proliferating line of cultured human cells (normal human liver with doubling time of 24 hr) was allowed to reach the stationary or plateau phase of growth, the initiation of DNA synthesis was also delayed by a single dose of radiation. This effect was not observed in several lines of human cells irradiated with 1000 rads (cGy) during exponential growth.

The effects of radiation on slowly proliferating cells in many normal tissues in vivo as well as for the “nongrowth” (G₀) fraction in tumors. Most malignant tumors are believed to contain a variable population of G₀ in addition to the rapidly proliferating growth fraction. It is also known that most of the cells in a number of normal mammalian tissues remain for longer periods of time in the G₁ phase of the cell cycle. G₀ cells are radioresistant.

The differential effect of X-irradiation on the slowly vs. rapidly dividing cells may be of particular importance in fractionated or prolonged X-ray exposure. Such a course of irradiation would progressively tend to block cells of a slowly proliferating population in G₁, in which a high proportion of the cells were already located before irradiation, while those in a rapidly proliferating population would proceed normally through this portion of the cycle. As a result, the distribution of cells within the cell cycle in each population would increasingly differ, leading to a progressive change in the radiation response of these cell populations with respect to one another. The survival after a fractionated exposure could, therefore, considerably differ between the two populations of cell, whereas the survival after a single exposure might be quite similar in each case.

III. EFFECT OF RADIOMIMETIC AGENTS

Nitrogen mustard, myleran, and dimethylmyleran are important radiomimetic agents. They are called radiomimetic because they mimic the effect of ionizing radiation. Like radiation, they can cause mutation, cancer, chromosomal damage, and cell death. These effects are due

to the fact that the radiomimetic agents alkylate the biological molecules. There are, however, many sites available for alkylation, and it is possible that different alkylating agents may show a different pattern of response.

Nitrogen mustard and dimethylmyleran produce very different patterns of response in leukemic cell cultures. Nitrogen mustard immediately stops cell division while dimethylmyleran allows cells to divide at least once before growth stops.

IV. SUMMARY AND COMMENTS

Radiation produces damage by ionization and free radicals. Low LET radiations such as X- and γ -rays produce damage primarily by free radicals, whereas high LET radiations such as α particles and protons produce damage primarily by ionization. The damage produced by neutrons is actually caused by protons. The damages produced by low LET radiations are modifiable, whereas the injuries produced by high LET radiations are not modifiable. When mammalian cells are irradiated *in vitro*, two types of survival curves, exponential and sigmoid, are obtained. The interpretations of these curves on three models have been discussed. The various concepts of radiosensitivity of mammalian cells were presented. Although the proposed concepts of radiosensitivity are applicable to normal cells and certain tumor cells, these concepts are inadequate to explain the variations in radiosensitivity that are commonly observed from one tumor type to another during radiation treatment of the same tumor. Therefore, the biochemical basis of radiosensitivity of tumor cells should be further investigated.

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Chapter 5

MODIFICATIONS OF CELLULAR RADIATION DAMAGE

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I. INTRODUCTION

There are two main purposes for studying the modification of radiation damage: (1) to obtain more information regarding the mechanism of radiation damage, and (2) to apply the knowledge of radiation biology to radiation therapy. During recent decades, the modification of the radiation effect *in vitro* and *in vivo* by various physical, chemical, and biological agents has been extensively studied. These agents have been reviewed.¹⁻³

II. PHYSICAL MODIFICATION

The shape of the survival curve depends upon several factors such as type of cells, oxygen, LET, and dose rate.

A. EFFECT OF OXYGEN

One of the most general and best-known modifying agents of radiation damage is molecular oxygen. Its ability to potentiate radiation response is called the oxygen effect, which is expressed in terms of the oxygen enhancement ratio (OER):

$$\text{OER} = \frac{\text{Dose under anoxic condition to produce an effect}}{\text{Dose under oxygenated condition to produce the same effect}}$$

It should be pointed out that the rate of oxygen utilization by cells is of no importance in the oxygen effect. The amount of molecular oxygen present in the cells is most important in increasing radiosensitivity. The value of the oxygen concentration for a midway response has been estimated to be about 8.5 $\mu\text{mol/liter}$ for cultured cells derived from human liver. The relative radiosensitivity increases as a function of oxygen concentration (Figure 5.1). The oxygen effect in cultured human liver cells has shown to be as follows:⁴

$$|D_0|_{\text{O}_2} = 119 \text{ rads (1.19 Gy)}$$

$$|D_0|_{\text{N}_2} = 263 \text{ rads (2.63 Gy)}$$

$$\text{OER} = \frac{263}{119} = 2.2$$

OER values from 1.2 to 3.1 have been reported; the reason for such a wide difference in the OER is unknown. All damage not produced by direct ionization of the molecules will be

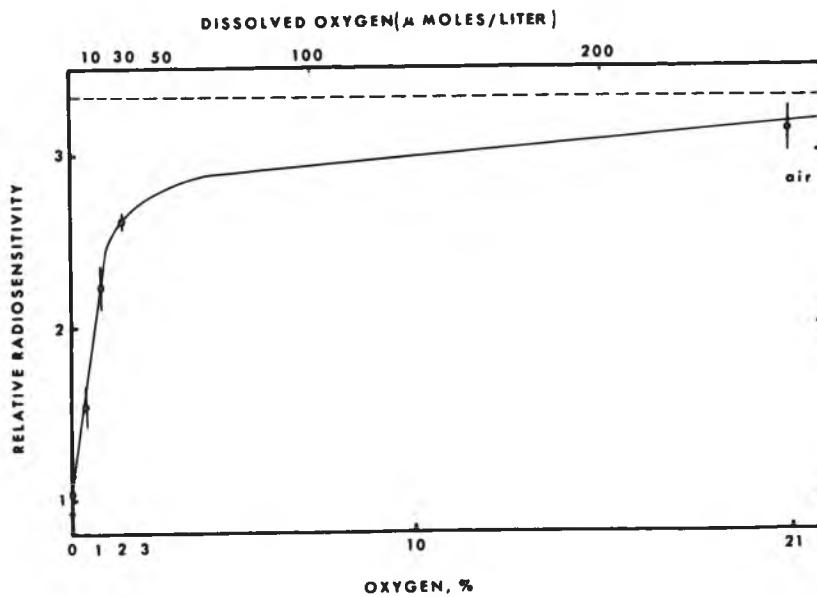


FIGURE 5.1. Typical variation in relative radiosensitivity of cells as a function of oxygen concentrations.

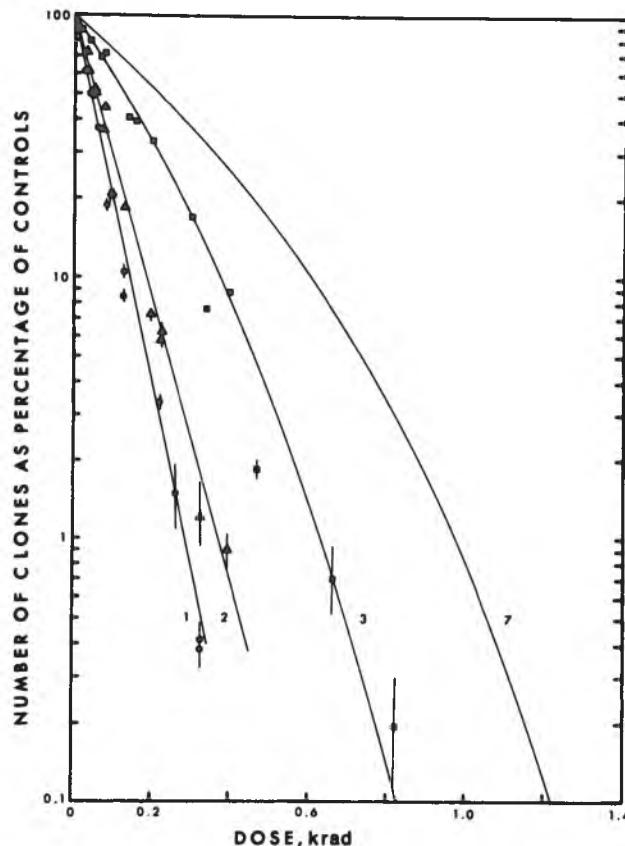


FIGURE 5.2. Survival of cultured cells derived from human kidney: Curve 1, 5.2 MeV α particles (LET 85.5, keV/ μ m); curve 2, 8.3 MeV α particles (LET 60.8 keV/ μ m); curve 3, 26.8 MeV α particles (LET 24.6 keV/ μ m); curve 7, 200 kV X-rays (average LET 2.5 keV/ μ m). Uncertainties are standard deviations. (From Barendsen, G.W., Walter, H.M.D., Fowler, J.F., and Bewley, D.R., *Radiat. Res.*, 18, 106, 1963. With permission.)

enhanced by the presence of oxygen. Because the presence of oxygen allows the formation of additional kinds of free radicals such as HO_2 (hydroperoxy free radicals) and RO_2 (peroxy free radicals), this may, in part, account for the enhanced damage of cells in the presence of oxygen.

B. EFFECT OF LET AND DOSE RATE

The dose-response curve for high LET radiation is more nearly exponential⁵ than for low LET (Figure 5.2). The effect of a high dose rate of low LET radiation is similar to that of any dose rate of high LET radiation; in other words, with a higher dose rate, the damage is greater. However, it should be pointed out that a difference in the biological response is not obvious until the two dose rates are widely different. The study of dose rate effect involves two different exposure times. If there is a difference in the biological effect following two different dose rates, it is not possible to decide whether this is due to the time of exposure. Because the repair process starts simultaneously with radiation damage, the time factor is indeed important in the final outcome of radiation injuries. This is true at a lower or intermediate range of dose rate, at which repair of radiation damage is possible. However, at a very high dose rate in which the repair process is minimal because of damage due to direct action, the time period may not appreciably influence the outcome of radiation damage.

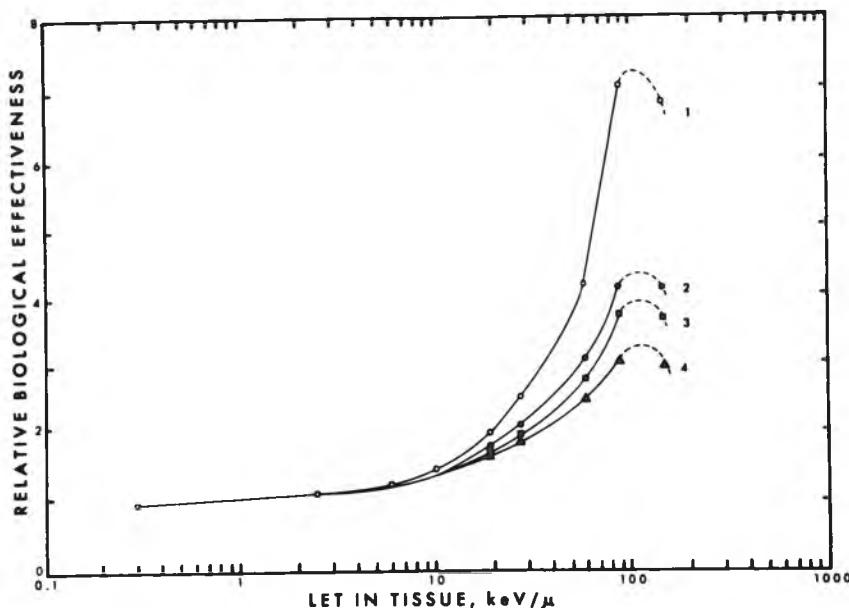


FIGURE 5.3. RBE of different radiations estimated from ratios of doses at the same survival level: Curve 1, 80% survival; curve 2, 20% survival; curve 3, 5% survival; and curve 4, 0.5% survival. (From Barendsen, G.W., Walter, H.M.D., Fowler, J.F., and Bewley, D.R., *Radiat. Res.*, 18, 106, 1963. With permission.)

C. OXYGEN EFFECT AND HIGH LET

Because damage produced by radiation of high LET is mostly due to a direct effect, the OER decreases as a function of LET. The OER for X-irradiated cultured human kidney cells is about 2.0, whereas it is about 1.1 when irradiated with α particles.⁵

D. RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)

The efficacy of different types of radiation⁶ to produce the same effect varies markedly. Therefore, a study of the RBE for each type of radiation is important.

$$\text{RBE} = \frac{\text{Dose to produce an effect with X-rays}}{\text{Dose to produce the same effect with radiation under investigation}}$$

Work already published on the different cell types has established the following general principles regarding RBE:

1. The RBE value for the same effect varies from one type of radiation to another.
2. The RBE value for the same effect and the same type of radiation differs if the radiation is delivered at markedly different energy levels, e.g., fast neutrons vs. slow neutrons.
3. The RBE value changes if the criterion of damage is changed.

The RBE value increases as a function of LET.² The RBE value peaks at about 100 keV/ μ m in tissues and differs at the different levels of survival (Figure 5.3).

III. CHEMICAL MODIFICATION

The chemical agents that modify the radiation in mammalian cells *in vitro* and *in vivo* can be grouped as either radioprotectors or radiosensitizers.

A. RADIOPROTECTORS

Radioprotectors are substances or procedures that significantly decrease radiation damage when added to the culture medium before irradiation. These agents do not reduce radiation injury when given after X-irradiation. The efficacy of radioprotectors *in vitro* differs markedly from that of radioprotectors *in vivo*. For example, some radioprotectors that are very effective *in vitro* have no appreciable effect *in vivo*, and vice versa. Some examples are given below.

1. Sulphydryl Compounds

Among sulphydryl (SH) compounds, cysteine, β -mercaptoethylamine (MEA, more commonly called cysteamine), and aminoethylisothiourea dihydrobromide (AET) have been most extensively studied. The efficacy of radioprotective substances is expressed in terms of the dose reduction factor (DRF), which is calculated as follows:

$$\text{DRF} = \frac{\text{Dose to produce an effect in the presence of radioprotector}}{\text{Dose to produce the same effect in the absence of radioprotector}}$$

It has been shown⁴ that cysteamine present during X-irradiation protects synchronized Chinese hamster cells in culture against lethal damage at all stages of the cell cycle. The effect is greatest for cells irradiated at sensitive phases such as mitosis, G₁, or G₂, and least for resistant cells in the S phase.⁷ For example, the DRF for G₁ equals 4.2 and for S equals 2.7. A 50 mmol concentration of cysteamine abolishes the age-dependent variability in the radiation response of cells. These agents are very toxic in humans and do not provide any differential protection between normal and tumor tissues. Therefore, they are of no clinical value during radiation therapy.

The mechanisms of radiation protection of these compounds are complex and usually involve more than one mechanism. The following mechanisms of radiation protection have been suggested for sulphydryl (MEA and AET) and other compounds.^{1,8}

"Free-radical scavenger" hypothesis: Several radioprotective SH compounds protect mammalian cells by trapping free radicals that are produced by the ionizing radiation.

Hypoxia: Some radioprotective SH compounds remove molecular oxygen from the solution and thereby produce hypoxic conditions during exposure. Hypoxia is a well-known condition for reducing radiation damage.

Mixed disulfide mechanism: This mechanism has been specifically postulated for the sulphydryl compounds, such as cysteamine and AET. It is suggested that an SH compound forms a temporary S–S bond with the enzyme containing the SH in the cells. This bond protects the sulfur atom of the enzyme against the indirect action of radiation; therefore, reconstitution of the functional SH group of the enzyme becomes possible after exposure. The weakest point of this hypothesis is that it assumes protein to be the most radiosensitive target within the cell. However, it is generally believed that DNA is the most sensitive spot in the cells.

Physiological shock mechanism: It has been suggested² that one of the mechanisms of protection by SH compounds is the production of reversible physiological shock. This is evidenced by the swelling of mitochondria after the treatment of cells by cysteamine. This structural alteration persists during the time the drug is effective in protecting the cells against the radiation damage.

Reversible inhibition of DNA synthesis: Several radioprotective agents reversibly inhibit DNA synthesis. For example, cysteamine depresses DNA synthesis and mitotic activity, which are reversible. It is presumed that an inhibition of DNA synthesis may delay replication and thereby increases the probability of DNA repair. If DNA synthesis continues on schedule, the DNA strands that are broken at several spots by irradiation will fall apart, leading to cell death. From the data available, it appears that the mechanism of protection is a very complex process and that more than one mechanism is involved for a given compound.

2. Cyclic Nucleotides

Cyclic nucleotides, adenosine 3',5'-cyclic-monophosphate (cAMP), and guanosine 3',5'-cyclic-monophosphate (cGMP) occur in all mammalian cells, and they are formed by catalyzing adenosine triphosphate (ATP) and guanosine triphosphate (GTP) by adenylate cyclase and guanylate cyclase, respectively. cAMP and cGMP are degraded by cAMP phosphodiesterase and cGMP phosphodiesterase, respectively. Numerous studies have shown⁹⁻¹¹ that cyclic nucleotides are involved in the regulation of growth, differentiation, and malignancy of certain cell types. Since the rate of proliferation and the degree of differentiation are important factors in determining the radiosensitivity of mammalian cells, the obvious question was whether cyclic nucleotides would modify the radiation response of normal cells or tumor cells.

In 1972, it was reported¹² for the first time that prostaglandin E₁ (PGE₁, a stimulator of adenylate cyclase) and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (R020-1724, an inhibitor of cyclic nucleotide phosphodiesterase) increased the survival of X-irradiated Chinese hamster ovary (CHOK₁) cells by about twofold, provided they were given before irradiation (Table 5.1). Both PGE₁ and R020-1724 were ineffective when given after irradiation. Based on this study, it was postulated that the intracellular level of cAMP may be inversely related to the radiosensitivity of mammalian cells.¹² Although the intracellular level of cAMP was not measured in the initial study,¹² other investigators have shown that the level of cAMP in CHO cells increases after treatment with PGE₁ and inhibitors of cyclic nucleotide phosphodiesterase.¹³ The basic observation on the radioprotective role of cAMP has been confirmed and extended by several investigators. For example, it was shown that cAMP-stimulating agents, when given before X-irradiation, increased the survival of irradiated Chinese hamster V-7914-15 and thymocyte¹⁶ cells in culture. It was also reported that cAMP-stimulating agents reduced X-ray-induced mitotic delay in human kidney T cells¹⁷ and in S-

TABLE 5.1
Effect of Prostaglandin (PG) E₁ and Inhibitor of Cyclic AMP Phosphodiesterase on the Survival of Irradiated CHO Cells

Treatment	Number of Colonies Formed			
	Preirradiation		Postirradiation	
	1 hr	4 hr	1 hr	4 hr
505 rads + solvent	28.0 ± 5.7*	33.0 ± 2.6	29.0 ± 3.0	32.0 ± 2.0
505 rads + PGE ₁ (10 µg/ml)	53.0 ± 4.6	55.0 ± 5.6	32.0 ± 3.0	33.0 ± 2.1
505 rads + R020-1724 (200 µg/ml)	55.0 ± 4.0	50.0 ± 3.5	30.0 ± 1.4	32.0 ± 3.0

Note: 400 cells were plated in Falcon dishes (60 mm) and prostaglandin E₁ and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (R020-1724) were added separately to each culture dish 24 hr after plating and for a period of 1 or 4 hr before irradiation. These agents were also added separately immediately after irradiation for a period of 1 or 4 hr. After incubation in the presence of various drugs, cells were washed and fresh growth medium was added. Irradiated control cultures were treated with an equal volume of solvent. The nonirradiated control cultures were treated with the same concentration of each drug and for the same period as the irradiated experimental cultures. Another set of nonirradiated control cultures received no treatment. The number of colonies in various nonirradiated and irradiated cultures was counted 12 days after irradiation. Each value represents an average of 6 to 8 samples.

* Standard deviation.

180 ascites tumor cells.¹⁸ Again, most of these studies did not assay the level of cAMP in the cells before irradiation. It was further suggested¹⁴ that changes in cAMP during the cell cycle may be one of the important factors responsible for the variation in radiosensitivity as a function of stages of cell cycle. In one study,¹⁵ it was shown that cells with an increased level of cAMP became more sensitive to irradiation at higher radiation doses. The exact reasons for this are unknown.

It was reported¹⁹ that ATP, 5'-adenosine monophosphate (5'-AMP), and 3'-adenosine monophosphate (3'-AMP), when given singly before irradiation, provided a significant degree of protection in irradiated mice. cAMP by itself was ineffective, but in combination with ATP it provided a higher degree of protection (70%) than that obtained with ATP alone (44%). The combination of 3'-AMP with 5'-AMP produced 100% survival.¹⁹ From these data it was suggested that a number of radioprotective agents mediate their effects via cAMP. However, it should be pointed out that exogenous cAMP generally does not penetrate the cell membrane. In addition, it is rapidly hydrolyzed to 5'-AMP in plasma. Similarly, because exogenous ATP does not generally enter the cell membrane, the radioprotective properties of the above agents may have been mediated by an indirect effect of these compounds.

Two studies have shown the involvement of cAMP in the mechanisms of radiation protection *in vivo*; for example, dibutyryl cAMP protected hair follicles²⁰ and gut stem cells^{20,21} against radiation-induced cell death without protecting lymphosarcoma and breast carcinoma.²⁰ This is a very exciting observation, because this is one of the very few examples in which a physiological substance protects normal tissue without protecting the tumor tissue. If similar results are observed in human tissue, an elevation of the intracellular level of cAMP during radiation therapy may improve the effectiveness of radiation therapy by protecting the normal tissue. It is well known that the damage to normal tissue becomes the limiting factor for the continuation of radiation therapy.

It is also well known that when the cells become hypoxic, they become radioresistant. It has been reported²² that the intracellular level of cAMP in moderately hypoxic cells markedly increases; therefore, the radioresistance of hypoxic cells may, in part, be related to a rise in cellular cAMP. It also appears that the radioprotective effect of SH compounds may, in part, be mediated by cAMP. For example, cysteamine, a well-known protective agent, increased the intracellular level of cAMP in the liver 15 min after administration.²³ However, the level of cAMP in proliferating tissue after administration of cysteamine was not measured. Therefore, one could not be certain whether the rise in the cAMP level of proliferating tissue was associated with radiation protection. Since cysteamine is known to produce hypoxia, the rise in the intracellular level of cAMP after treatment with cysteamine was expected. Preincubation of mouse bone marrow cells with PGE₁ — a stimulator of adenylate cyclase, cAMP, and cGMP — before the addition of cysteamine, resulted in an increase of the radioprotective effect of cysteamine.²⁴ Unfortunately, the intracellular level of cAMP was not measured under the above experimental conditions. In addition, the rationale for the addition of cAMP and cGMP was not provided.

Although some *in vitro* and *in vivo* data suggest that the level of cAMP may be one of the important factors in the radiosensitivity of mammalian cells, there has been no study on the role of cGMP on the radiosensitivity of mammalian cells. The availability of analogs of cGMP and stimulators of guanylate cyclase makes it possible to investigate this problem. Further *in vitro* and *in vivo* studies on the role of cAMP and cGMP in radiosensitivity of normal and tumor cells must be investigated.

B. EICOSANOIDS

In addition to prostaglandin E, other eicosanoids such as leukotriene-C₄ protects Chinese hamster fibroblasts *in vitro*,^{8,129} mouse hematopoietic stem cells *in vivo*,¹³⁰ and mouse intestinal crypt cells *in vivo*¹³¹ against radiation injury. DRF of whole-body irradiated mice is about

TABLE 5.2
Radioprotective Effect of Calcium
Antagonist, Diltiazem, against Whole-Body
X-Irradiation (8.5 Gy) in Mice^a

Drug Dose	% Survival at 30 Days
Irradiation alone	0
Irradiation + 110 mg/kg of body wt	93
Irradiation + 55 mg/kg of body wt	58
Irradiation + 27.5 mg/kg of body wt	17

^a Drug was given 15 min before irradiation. Data are summarized from a previous publication.¹²⁷

1.9.¹³² One of the mechanisms of radiation protection *in vivo* appears to be mediated via hypoxia¹³³ and scavenging of free radicals.¹³⁵

C. CYTOKINES

Interleukin-1 α administration increases the survival of irradiated mice.¹³⁴ Conversely, the injection of anti-interleukin-1 receptor antibody decreases the survival of irradiated animals. The optimally radioprotective doses of a combination of interleukin-1 α and tumor necrosis factor- α (TNF- α) result in additive radiation protection. In addition, the suboptimal doses of interleukin-1 α in combination with nonprotective doses of granulocyte-macrophage colony-stimulating factor or granulocyte colony-stimulating factor produce synergistic protection.¹³⁶

D. ONCOGENE AND CELLULAR GENES

Human normal fibroblasts transfected with mutated *ras* oncogene or SV40-T-antigen gene generate immortalized cells that express high levels of mutated *ras* oncogene or SV40-T-antigen gene. These immortalized cells become more radioresistant than their untransfected counterparts.¹³⁷ This acquired radioresistance may be due to a prolonged cell-cycle delay following irradiation of cells.¹³⁸

E. OTHER PHARMACOLOGICAL AGENTS

Cimetidine, an antagonist to the histamine H₂-receptor — which is commonly used in the treatment of peptic ulcers — is a good radioprotective agent in mice.¹³⁹ DRF = 1.5. The proposed mechanism includes free radical scavenging.

F. AS101 [AMMONIUM TRICHLORO(DIOXYETHYLENE-O-O') TELLURATE]

AS101 is a new synthetic immunomodulating compound which stimulates the production of cytokines. It acts as a radioprotective agent in mice.¹⁴⁰ It protects primarily hematopoietic systems against radiation damage. It is unknown whether AS101 can prevent tumor cells *in vitro* or *in vivo* against radiation damage.

G. CALCIUM ANTAGONIST

A calcium antagonist, diltiazem, with a benzothiazepene structure, appears to be a good radioprotective agent in whole-body irradiated female mice (Table 5.2). This agent also acts as a radiation therapeutic agent, because it increases the survival of irradiated mice from 0 to 42% when given after X-irradiation.¹²⁷ Other calcium antagonists, such as nifedipine, nimodipine, isradipine, and nitrendipine, also act as radioprotective agents from 25 to 58%.

Diltiazem, in combination with zinc aspartate, dimethyl sulfoxide (DMSO), and nifedipine, produces synergistic effects on radiation protection.¹²⁸ The mechanisms of protection may involve inhibition of cellular calcium overload, which occurs following radiation-induced membrane damage. Some calcium antagonists, such as diltiazem and nifedipine, act as antioxidants; therefore, the mechanisms of radioprotection may involve scavenging of free radicals. It is unknown whether the calcium antagonist protects only the normal tissue and the tumor tissue against radiation damage.

1. WR-2721 [S-2-(3-Aminopropylamino)-ethylphosphorothioic Acid]

It has been reported^{49,50} that WR-2721 protects normal tissue, but does not protect tumor tissue, against radiation damage. It also protects normal tissue against damage produced by alkylating agents. WR-2721 does not protect the brain or spinal cord.⁵⁰ Preliminary data⁵¹ show that relatively long tumor regression can be obtained in some dogs with spontaneous tumors when treated with radiation combined with WR-2721. However, this study failed to show an improvement in the therapeutic ratio. One dog died of cardiac arrest. One of the mechanisms of action of WR-2721 involves free radical scavenging. The radiation protection effect of WR-2721 is mediated by its dephosphorylated metabolite, WR-1065 [S-2-(aminopropylamino)-ethyl phosphorothioic acid], which is present as a free sulfhydryl compound. Clinical results in humans have been disappointing because of its toxicity.

The depletion of polyamines by difluoromethyl ornithine (DFMO), and of glutathione (GSH) by buthionine sulfoximine (BSO) increases the radiosensitivity of mammalian cells in culture.¹⁴¹ WR-1056 treatment protected glutathione-depleted cells by a factor of 1.98 and polyamine-depleted cells by a factor of 2.29, whereas it protected control undepleted cells by a factor of 2.09.¹⁴¹ WR-1065 also protects against radiation-induced mutation, cell transformation, and DNA strand scissions.¹⁴² This free thiol form of WR-2721 is more effective in providing radiation-induced chromosomal aberrations than DMSO. WR-1065 protects fission-neutron-induced transformation of fibroblasts by a factor of about three.¹⁴³

H. ANTIOXIDANT VITAMINS

Antioxidant vitamins, such as vitamin C, β-carotene, vitamin A, and vitamin E, modify the effect of X-irradiation on tumor and normal cells in a selective manner. For example, some preliminary data show that they may enhance the growth-inhibitory effect of X-irradiation on tumor cells, but protect normal tissue against radiation damage. These vitamins at high doses, when used individually, are generally cytotoxic for tumor cells but not for normal cells.

I. VITAMIN C

In vitro data suggest that vitamin C (sodium ascorbate) modifies the effect of X-irradiation on the survival of mammalian cells in culture. However, the extent of modification depends upon the type of cells. For example, sodium ascorbate increases the growth inhibitory effects of X-irradiation on mouse neuroblastoma cells in culture (Table 5.3), whereas it protects Chinese hamster ovary cells²⁶ but does not modify the effect of X-irradiation on rat glioma (C-6) cells in culture.²⁵ A study²⁷ has shown that vitamin C, when combined with ionizing radiation, increased the survival of mice with ascites tumor cells in comparison to those treated with X-irradiation alone. Dehydroascorbate, a metabolite of vitamin C, produced cytotoxic effects primarily on hypoxic Chinese hamster lung cells in culture, and sensitized the effect of ionizing radiation in Ehrlich ascites cells *in vivo*.²⁸ Because the hypoxic cells are known to be highly radioresistant, the addition of high doses of vitamin C during radiation therapy may be more effective in killing hypoxic cells by dehydroascorbate. The efficacy of this mechanism depends upon the proportion of hypoxic cells in the tumor mass at the time of vitamin C treatment.

TABLE 5.3
Effect of Sodium Ascorbate (Vitamin C) on
X-Ray-Induced Growth Inhibition in Murine
Neuroblastoma and Rat Glioma Cells in Culture^a

Treatment	Growth Inhibition (% of Control)	
	Neuroblastoma	Glioma
Sodium ascorbate (100 µg/ml)	105 ± 9 ^b	98 ± 5
X-irradiation (400 rads)	28 ± 2	29 ± 3
Sodium ascorbate plus X-irradiation	1.8 ± 0.4	27 ± 3

^a Sodium ascorbate was added before X-irradiation and was present throughout the experiment. Data are summarized from our previous publication.¹⁵⁰
^b Standard error of the mean.

Vitamin C protected normal tissue against adverse effects of X-rays, but it did not modify X-ray-induced tumor growth inhibition.¹⁴⁴ Administration of 5 g vitamin C at 4-hr intervals failed to modify the adverse effects of radiation after completion of radiation therapy.¹⁴⁵ In addition, local application of 10% vitamin C failed to prevent radiation-induced dermatitis in humans.¹⁴⁶ To evaluate the role of vitamin C in radiosensitivity, a well-designed clinical trial that considers stability, biological turnover, doses, and appropriate time of administration is essential before the efficacy of vitamin C in modifying radiation damage on tumor cells and normal cells is evaluated.

J. VITAMIN A AND β -CAROTENE

Pretreatment of animals with retinol increases the growth-inhibitory effect of X-irradiation on mammary adenocarcinoma, L1210 leukemia, and a fibrosarcoma.²⁹⁻³² The administration of vitamin A alone did not affect the growth of these tumors. Retinol also causes inhibition of DNA repair processes in UV-irradiated lymphocytes.³² This suggests that vitamin A-induced enhancement of the effect of X-irradiation on tumors may be due to the inhibitory effect of retinol on DNA repair processes.³³ It has been reported¹⁴⁷ that all-*trans*-retinoic acid (RA) inhibits the repair of potential lethal damage more effectively in human breast cancer cells (MCF-7) than that in normal human fibroblasts (AG1522). RA is also a more effective radiosensitizer for MCF-7 cells than for normal fibroblasts (Figure 5.4).

β -Carotene administration reduces the severity of radiation-induced mucositis in humans.¹⁴⁸ Dietary supplementation with vitamin A or β -carotene enhances the growth-inhibitory effects of X-irradiation selectively on transplanted breast adenocarcinoma in mice and protected the normal tissues against some of the damaging effects of irradiation (Table 5.4).¹⁴⁹ Additional studies are needed to confirm the role of vitamin A and β -carotene in modifying radiation response of tumor and normal cells.

K. VITAMIN E

The studies on the radioprotective effect of vitamin E have produced conflicting results.³⁴⁻³⁶ The primary reasons for these conflicting results are differences in doses of vitamins, type of vitamin E, doses of radiation, and administration time. The experimental results of various studies are listed in Tables 5.5 and 5.6. Very few of these studies have measured the cellular level of vitamin E before or after irradiation. There are different forms of vitamin E, such as α -tocopheryl acetate (α -TA), α -tocopheryl nitotinate (α -TN), α -tocopheryl acid succinate (α -TS), and α -tocopherol (α -T). The stability and the solubility of these forms are very different. α -TS appears to be the most effective form of vitamin E in the tissue culture system. Also,

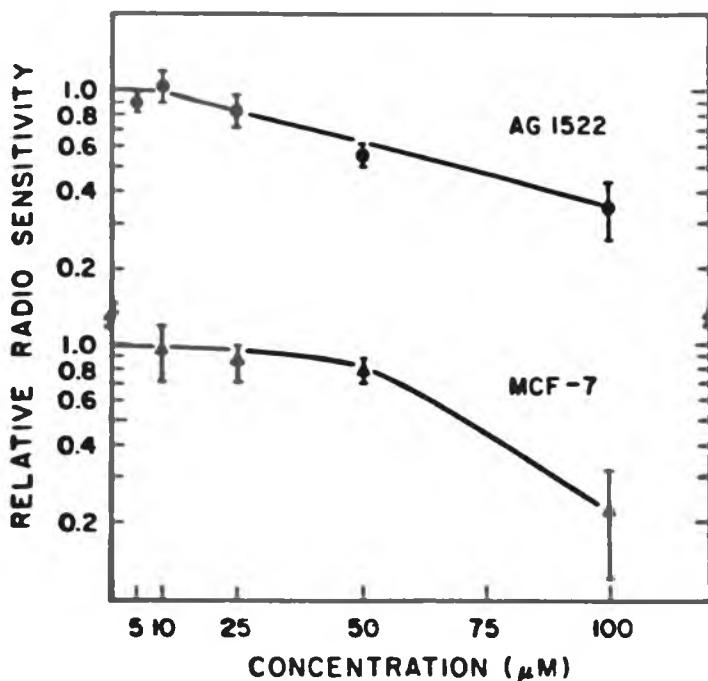


FIGURE 5.4. Effects of all-*trans*-retionic acid on radiosensitivity on human breast cancer cells (MCF-7) and normal human diploid fibroblasts (AG1522). (From Rutz, H.P. and Little, J.B., *Int. J. Radiat. Biol.*, 16, 1285, 1989. With permission.)

the natural form (d) of vitamin E is more effective than the synthetic form (DL). Therefore, it is very important that the radioprotective effect of various doses of each form of vitamin E be systematically investigated.

In vitro studies suggest that the presence of α -tocopherol during irradiation reduces the radiosensitivity of erythrocyte membranes;^{47,48} vitamin E, when given after irradiation, has no effect. Dietary vitamin E treatment significantly reversed radiation-induced immunosuppression as measured by the delayed-type hypersensitivity response to oxazolene.³⁴ These studies suggest that the presence of this vitamin modifies the response of radiation at several levels. α -TS enhances the effect of X-irradiation on some tumor cells, such as murine neuroblastoma

TABLE 5.4
Effect of Supplemental Vitamin A and β -Carotene and Local X-Irradiation on Survival of Mice with Transplanted Breast Adenocarcinomas

Treatment	Number of Mice Survived after 1 Year
Control with transplanted tumor	0/24
3000 rads (30 Gy), single dose	0/24
Vitamin A (3000 IU/mouse)	0/24
β -Carotene (270 $\mu\text{g}/\text{mouse}$)	0/24
Vitamin A + 3000 rads	22/24
β -Carotene + 3000 rads	22/44

Note: Data are summarized from the work of Seiffer et al.¹⁴⁹

TABLE 5.5
Modification of the Effects of Ionizing Radiation by α -Tocopherol and Its Ester Derivative in Whole Animals

Animals	Type	Dose (mg/kg)	Route	Injection Schedules	Radiation Dose (rad)	Conclusions
Mice	α -T ac	15	Oral	0.036% diet -1 week + 30 days	650, 750, 850	Increase in survival ³³
Rats	α -T	60	Oral	-2 to + 1 day	700	No effect on survival ³⁴
Mice	α -T	300	i.m.	-5 to + 5 days	700	Marginal increase in survival ³⁵
Male mice	α -T aq	25-150	i.m.	0 day and until death	550	Ineffective at lower concentrations; increases mortality at higher concentrations ³⁶
Male mice	Mixed α -T or α -t ac	0.25% diet 40-600	Diet i.p.	-6 weeks to + 4 months 0 day	1600 (200/week) 500-750	No effect on life span ³⁷
Female mice	α -T aq	1000	i.p.	0 day	400-800	Increase in survival at lower concentrations; no effect at higher concentrations ³⁸
Female mice	α -T	0.01% diet	Diet	Various periods pre- and postirradiation	800	Increased survival at 500, 600 rads ³⁹
Female mice	α -T aq	50	i.p.	Immediate post- irradiation (on deficient or normal diet)	800	No effect on survival ⁴⁰
Rats	α -T and α -T aq	2.5% diet	Diet	-2 weeks until irradiation and α -T aq 4 hr before irradiation	1500-2000	Increase in survival at lower concentration; no effect at higher concentrations ⁴²
Male mice	α -T	27, 55, 82	i.p.	-1 day	550, 650	Thoracic

Note: α -T = α -tocopherol; α -T ac = α -tocopheryl acetate; α -T aq = aqueous preparation; 0 day = 10-30 min before irradiation; (-) = before irradiation; (+) = after irradiation.

TABLE 5.6
Modification of the Effect of Ionizing Radiation by α -Tocopherol and Its Ester Derivative on Tumor Cells

Animals	Type	Dose (mg/kg)	Route	Injection Schedules	Radiation Dose (rad)	Conclusions
Mice with squamous cells	α -T aq	1000	i.p.	0 day	1000–2000 whole-body	Increased survival of tumor cells in α -T-treated ³⁹
Rats with i.m. tumors	α -T aq	50	i.m.	-7 or -1 day	3000 to tumor	Radiation sensitizing effect on α -T ac on tumors ⁴³
Rats with i.m. tumors	α -T ac or d- α -T ac	50–1000 37	i.m. Oral	-7 days	3000 to tumor	Sensitizing effect due to radiation up to 500 mg, but not with 1000 mg ⁴⁴
Mouse neuroblastoma cells in culture	α -T aq	5 μ g/ml		3 days	400	Enhances the inhibitory effect ⁴⁵

Note: α -T = α -tocopherol; α -T ac = α -tocopheryl acetate; α -T aq = aqueous preparation; 0 day = 10–30 min before irradiation; (-) = before irradiation; (+) = after irradiation.

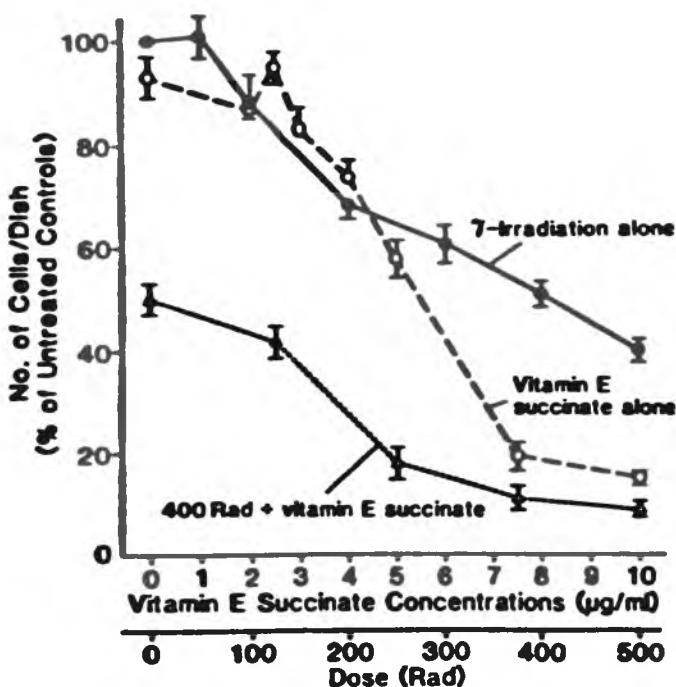


FIGURE 5.5. Neuroblastoma cells (NB_{P_2}) were plated in tissue culture dishes (60 mm), and the cells were γ -irradiated 24 hr after plating. Vitamin E succinate or the solvent (ethanol 0.25% and sodium succinate 5 $\mu\text{g}/\text{ml}$) was added immediately before irradiation. The drugs and medium were changed after 2 days of treatment. The number of cells per dish was determined after 3 days of treatment. Each experiment was repeated at least twice involving three samples per treatment. The average value ($172 \pm 7 \times 10^3$) of untreated control NB cells was considered 100%, and the growth in treated cultures was expressed as a percentage of untreated controls. The bar at each point is the standard error of the mean. (From Sarria, A. and Prasad, K.N., *Proc. Soc. Exp. Biol. Med.*, 175, 88, 1984.)

cells (Figure 5.5). However, a systematic study on the role of vitamin E in modifying the radiation response of tumor cells and normal cells is needed.

L. RADIOSENSITIZERS

Radiosensitizers are substances or procedures that increase the radiosensitivity of the cells, when they are present during or after irradiation.

1. Analogs of Pyrimidine

The analogs of DNA bases are important radiosensitizers. Among the radiosensitizers, the halogenated pyrimidines have the greater interest in connection with mammalian cells. The synthesis of these drugs was motivated by their potential use in cancer therapy. The analogs of thymidine, 5-chlorodeoxyuridine (CldU, also referred to as CUDR), 5-bromodeoxyuridine (BrdU, also referred to as BUdR), and 5-iododeoxyuridine (IdU, also referred to as IUDR) incorporate into DNA instead of thymidine, whereas 5-fluorodeoxyuridine (FdU, also referred to as FUdR) inhibits *de novo* DNA synthesis by interfering with the action of thymidylate synthetase. FUdR forms fluorouracil (FU) after cleavage of the base or after oxidation of the sugar moiety; FU then can incorporate into RNA.

a. Effect on Human Bone Marrow in Culture

Radiosensitizing effects of analogs of DNA bases on human bone marrow cell culture (D98/AG) have been studied.⁵² After 4 days of growth in the presence of 0.004 $\mu\text{g}/\text{ml}$ of FdU

and $2 \times 10^{-6} M$ CldU, BrdU, or IdU, cells were X-irradiated and then plated for survival study. FdU was added to minimize *de novo* synthesis of thymidine, which then allowed incorporation of more analogs of DNA. IdU produced the largest potentiating effects of X-rays, but IdU was found to be the most toxic at concentrations higher than $2 \times 10^{-6} M$; however, BrdU was less toxic than IdU while still affording increasing potentiation of radiation damage with increasing concentration. The D_0 values of irradiated controls and irradiated and BrdU-treated cells are as follows: D_0 control = 180 R, and D_0 (BrdU $8 \times 10^{-5} M$) = 55 R. Thus, BrdU at a higher concentration increases the radiosensitivity more than 3-fold.

b. Mechanism of Action of Analogs of Thymidine

The exact mechanism of radiosensitization by the analogs of thymidine is unknown; however, two possible modes of action have been proposed:

1. Weakness of the sugar phosphate backbone of the DNA strand could result from electrostatic repulsion between the electronegative halogen atom and the neighboring electronegative phosphate group. The presence of these weakened backbone structures is assumed to make DNA more sensitive to radiation damage.
2. Interference with the repair mechanism. Radiosensitizing agents may interfere with the repair mechanism within the cells after irradiation.

2. Analogs of Purine

Purine analogs such as 6-mercaptopurine, 2-aminopurine, and 6-thioguanine are also powerful radiosensitizers. However, these compounds are not as selective as the pyrimidine analogs because they enter into both DNA and RNA. In addition, the analogs of purine have the following limitations: (1) radiosensitization is oxygen-dependent, and (2) the compounds are highly toxic. These limitations are not observed with the analogs of pyrimidine.

3. Actinomycin D

Actinomycin D is known to inhibit mRNA synthesis in mammalian cells. In HeLa cells, a short pre-exposure treatment (75 min) with actinomycin D ($1.0 \mu\text{g/ml}$) appears to potentiate the radiation response, which is dose-dependent. However, as the drug-enhanced response increases, the relative plating efficiency decreases.

4. Hydroxyurea

Hydroxyurea kills cells in the S phase of the cell cycle and blocks the cells at the end of the G₁ phase, thereby partially synchronizing the cells. Because the G₁ cells are relatively radiosensitive, the radiation response of cells in the presence of hydroxyurea markedly increases.

5. Electronaffinic Compounds

During the last 20 years, new compounds belonging to the electronaffinic class have been developed to increase the radiosensitivity of hypoxic tumor cells,⁵³ which are generally resistant to low LET radiations (X- and γ -rays). The number of hypoxic cells in human tumors varies markedly from one type to another. Thomlinson et al.⁵⁴ estimated $\geq 12\%$ for sarcomas and 12–20% for carcinomas. Ash et al.⁵⁵ estimated 1–20% for sarcomas and 50–70% for carcinomas; and Denekamp et al.⁵⁶ estimated 50–80% for sarcomas and 10–35% for carcinomas. The most extensively used electronaffinic compounds include (2-nitroimidazole-1-yl)-3-methoxy-2-propanol (misonidazole, RO-07-0582); desmethylmisonidazole (RO-05-9963), 5-nitromidazoles (metronidazole and nimorazole), and nitrofuran. These compounds belong to the electronaffinic class because of the direct relationship between radiation sensitization ability and the electron affinities of the compounds.

TABLE 5.7
Effect of Electronaffinic Compounds on Radiation Response
of B-16 Melanoma Tumors in Mice (C57BL)^a

Agent	Doses (mg/kg)	Sensitizer Enhancement Ratio (SER)
Misonidazole	1000	1.8
Desmethylmisonidazole	1000	1.7

Note: Agents were administered i.p. 1 hr prior to irradiation. The tumor-bearing flank was irradiated with a single dose of 1000–3000 rads. Sensitizer enhancement ratio (SER) was calculated as follows: dose of radiation alone to produce a regrowth of 9 days/dose of radiation plus agent to produce the same effect.

^a These data are summarized from Clement, J.J., Wodinsky, I., Johnson, R.K., and Silveria, D.M., in *Radiation Sensitizers*, Brady, L.W., Ed., Masson, New York, 1980.

Of the various electronaffinic compounds, misonidazole has been studied most extensively in both laboratories and clinics. Therefore, the effects of this compound will be discussed in detail. Misonidazole has been known to produce the following effects: (1) radiosensitization of hypoxic tumor cells,⁵⁷ (2) direct killing of hypoxic tumor cells,⁵⁸ (3) radiosensitization of certain normal tissues,⁵⁹ (4) neurotoxicity,⁶⁰ (5) mutagenicity to bacterial as well as mammalian cells under oxic and hypoxic conditions,^{61–62} and (6) oncogenic transformation *in vitro* and *in vivo*.⁶³ The sensitizer enhancement ratio (SER) for mammalian hypoxic cells *in vitro* varies from 1.2 to 2.4.^{64–68} The SER⁶⁹ of misonidazole and desmethylmisonidazole on B-16 melanoma cells is listed in Table 5.7. Misonidazole also sensitizes the effect of irradiation on hypoxic tumor cells at a low dose rate.⁷⁰ The effect of misonidazole and other electronaffinic compounds on the frequency of transformations in comparison to ionizing radiation is described in Table 5.8. The frequency of transformations produced by X-irradiation and misonidazole is similar; however, the frequency of transformations produced by neutrons is twofold higher than that produced by X-irradiation or by misonidazole treatment.⁶³

The primary toxic effect of misonidazole is neurotoxicity. The extent of neurotoxicity depends upon drug dose and ranges from mild sensory neuropathies of the hands and feet to

TABLE 5.8
Effects of Electronaffinic Compounds and Ionizing
Radiation on Mouse Embryo Fibroblasts (10T_{1/2})^a

Agents	Frequency of Transformation per Surviving Cell
Misonidazole (1 mM)	0.023
Desmethylmisonidazole (1 mM)	0.027
RO-0741 (1 mM)	0.046
SR-2508 (1 mM)	0.110
X-rays (100 rads)	0.025
Neutrons (200 rads)	0.060
Neutrons (50 rads)	0.023

^a The data are summarized from Miller, E.C. and Hall, E.J., in *Radiation Sensitizers*, Brady, L.W., Ed., Masson, New York, 1980.

TABLE 5.9
Incidence of Toxicity in Humans Treated with
Misonidazole and Irradiation

Toxicity	% of Treated Patients
Peripheral neuropathy	20-25
Central neuropathy	9
Gastrointestinal (nausea and vomiting)	58

convulsion.^{71,72} The types and incidence of toxicity of a U.S. series⁷⁶ are listed in Table 5.9. The neurotoxicity of the electronaffinic compounds has become a limiting factor in their use in the radiation therapy of human tumors.

Mechanisms of the actions of misonidazole: Cysteamine protects against the misonidazole-induced cytotoxicity⁷⁴ as well as the radiosensitization of hypoxic cells.⁷⁵ Based on these observations, it was suggested⁷⁶ that misonidazole depletes nonprotein thiols from hypoxic cells. Another mechanism suggests⁷⁷ that binding of nitroreduction products of misonidazole to cellular macromolecules may be involved in the cytotoxicity of misonidazole. Although cysteamine protects against misonidazole-induced radiosensitization of hypoxic tumor cell in culture,⁷⁵ sodium ascorbate enhances the cytotoxic effect of misonidazole.⁷⁸

Clinical trials of misonidazole: A recent study⁷⁹ has reported the results of clinical trials of misonidazole using over 200 patients. Neurotoxicity, primarily peripheral neuropathy, was observed in 28% of those given two or more doses (12 g/m²). Randomized clinical trials in carcinoma of the bronchus, bladder, and breast have shown no advantage.⁷⁹ Preliminary results suggest that misonidazole may be of some value in improving radiation therapy in carcinoma of the cervix.⁷⁹

Combination of hyperbaric oxygen and misonidazole: 13 of 19 untreated patients with squamous carcinomas are locally clear of disease after a combined treatment of hyperbaric oxygen and misonidazole.⁸⁰ In cancer of the head and neck, 20 of 32 patients were locally free of disease.⁸⁰ Compared with retrospective controls.⁸⁰

Preliminary results of a randomized clinical trial of low doses of misonidazole in patients with high-grade astrocytoma appear to be favorable for the group receiving misonidazole,⁸¹ with a minimum follow-up time of 1 year. The absolute survival time was increased at least by 3 months. Misonidazole failed to improve radiation therapy of glioma tumors.⁸² Complete tumor regression 3 months after irradiation was obtained in 18 of 22 patients.⁸³ Reversible peripheral neuropathy was observed in 5 of 22 patients.⁸³ In the U.S. series, 20–25% of the patients receiving radiation and misonidazole developed peripheral neuropathy and 58% gastrointestinal toxicity, which included nausea and vomiting; 9% developed central neuropathies, which primarily included confusion, lethargy, and a decrease in mental functions. Preliminary data from the phase II clinical trials suggest that no significant improvement was observed by the addition of misonidazole in radiation therapy protocol.⁷³

The clinical results of hypoxic cell sensitizers have been disappointing because of their toxicity to neurons and the GI tract. Nevertheless, efforts are being made to synthesize new groups of electronaffinic compounds that can accumulate in human tumor tissue in larger amounts and produce fewer side effects.

Among newly developed hypoxic cell radiosensitizers, fluorinated 2-nitroimidazole derivatives, KU-2285, is an effective radiosensitizer *in vitro* and *in vivo* at both high and low radiation doses.¹⁵¹ The sensitization enhancement ratio (SER) for 100–400 mg/kg of KU-2285 was between 1.12 and 1.42. RK-28, a 2-nitroimidazole nucleoside analog, is another newly developed hypoxic cell radiosensitizer.^{152,153} Pimonidazole and etanidazole are two recently

developed hypoxic cell radiosensitizers.¹⁵⁴ The clinical results of most of these radiosensitizing agents remained disappointing.

6. Hyperthermia

In 1893, Coley demonstrated for the first time that the induction of high temperatures in cancer patients with bacterial toxins caused tumor regression.⁸⁴ Since then, there have been numerous reports of cases of arrest or total disappearance of cancer after prolonged high fever.⁸⁵ The reports⁸⁶⁻⁸⁸ that hyperthermia can sensitize the effect of irradiation on hypoxic cells led to an extensive study on this topic, because of the clinical implications.

Experimental hyperthermic temperatures used in various studies ranged from 41 to 45°C. There have been some excellent reviews on this subject.⁸⁹⁻⁹³ During the course of various studies, the phenomena of thermotolerance and thermosensitization were observed.⁹³⁻⁹⁸ Thermotolerance can be induced in three ways: (1) incubation for a short time at 43°C followed by incubation at 37°C, (2) prolonged incubation at 41.5–42.5°C, and (3) incubation at 38–41°C followed by incubation at 43–45°C. Thermosensitization can be induced by incubating the cells at higher temperatures (43–45°C), followed by incubation at lower temperatures (38–41°C). The mechanisms of induction of thermotolerance and thermosensitization are unknown.

Extensive tissue culture work led to the studies on animal and human models. Before discussing the studies on *in vivo* models, it is important to define the following terminologies:

$$\text{Thermal enhancement ratio (TER)} = \frac{\text{response dose of radiation alone}}{\text{response dose of radiation with hyperthermia}}$$

$$\text{Tumor control dose (TCD}_{50}\text{)} = \frac{\text{dose required for tumor control}}{\text{in 50% of mice with tumors}}$$

$$\text{Therapeutic gain factor (TGF)} = \frac{\text{TER tumor}}{\text{TER skin}}$$

There have been numerous experimental and some clinical studies to evaluate the role of hyperthermia in radiation therapy.⁹⁹⁻¹⁰⁷ One example⁹⁹ of such a study is described below, using C₃H mice mammary carcinoma. The following data were obtained. With simultaneous heating at 42.5°C for 60 min, using a one or five radiation fraction dose, TER for both tumor and surrounding skin was 2.5. No therapeutic gain was observed under the above experimental conditions. However, when hyperthermia was applied 4 hr after irradiation, a significant therapeutic gain factor was observed (TER tumor = 1.41, TER skin = 1.03, therapeutic gain factor = 1.37).

Hyperthermia has been shown to enhance the effects of misonidazole on animal tumor models.^{108,109} Since misonidazole is known to be neurotoxic, the potential significance of the above observation in the management of tumors remains to be seen.

Some studies show that hyperthermia increases the frequency of radiation-induced transformation in C₃H 10T1/2 cells in culture.^{110,111} The clinical studies have reported that systemic hyperthermia at high temperatures may cause peripheral and sensory neuropathy and convulsions; therefore, the use of such approaches has no value in the management of tumors. The experimental and clinical studies also reveal that hyperthermia in combination with radiation would be a unique tool in the local control of tumors.

Kim has published an excellent review on hyperthermic sensitizers in mammalian cells in culture.¹⁵⁵ Some of them are described below:

1. Membrane active agents: procaine, lidocaine and ethanol
2. Energy depleters: glycolytic inhibitors: 5-thio-D-glucose, lonidamine, and gossypol
oxidative phosphorylation inhibitor: rhodamine
lactate transport inhibitor: quercetin
3. Polyamines: spermidine
4. Thiol compounds: cysteamine and cysteine
5. cAMP stimulating agents: prostaglandin E₁, and an inhibitor of cyclic nucleotide phosphodiesterase
6. α -Tocopheryl succinate (vitamin E)

The role of cAMP in heat sensitization is not well defined. Kim et al.¹⁵⁵ have reported that dybutyryl cAMP acts as a hyperthermic protector, whereas Rama and Prasad have shown that an increase in intracellular levels of cAMP by prostaglandin E₁, or R020-1724, an inhibitor of cyclic nucleotide phosphodiesterase, increases heat sensitivity of neuroblastoma cells in culture.¹⁵⁶ More studies are needed to clarify the role of cAMP in heat sensitivity. α -Tocopheryl succinate (vitamin E succinate) sensitizes the effect of heat on neuroblastoma cells in culture (Table 5.10).

M. HYPERTHERMIC PROTECTORS

Some hyperthermic protectors are:

1. Deuterium oxide
2. Glycerol
3. Dybutyryl cAMP
4. Sodium butyrate
5. Retinoic acid

From these studies it appears that several agents, such as 5-thio-D-glucose, quercetin, cAMP, α -tocopheryl succinate, and retinoids, are important modifiers of heat sensitivity.

TABLE 5.10
Modification of the Effect of Hyperthermia on Neuroblastoma Cells in Culture by α -Tocopheryl Succinate^a

Treatments	Number of Cells/Dish (% of Treated Control)	Trypan Blue Stained Cells (% Attached Cells)
Sodium succinate plus ethanol (0.25%)	102 \pm 3 ^b	<1
43°C (20 min)	40 \pm 1	4
Vitamin E succinate (5 μ g/ml)	50 \pm 3	5
Vitamin E succinate ^c + 43°C	9 \pm 1	27
43°C + vitamin E succinate ^d	30 \pm 6	6
41°C (45 min)	56 \pm 3	1
Vitamin E succinate ^c + 41°C	21 \pm 2	12
41°C + vitamin E succinate ^d	32 \pm 2	6
Sodium succinate ^c + ethanol + 41°C	52 \pm 2	1

^a Data were summarized from a previous publication.¹⁵⁶

^b Standard error of the mean.

^c Vitamin E succinate was added before heat treatment and was present throughout the experiment.

^d Vitamin E succinate was added after heat treatment and was present throughout the experiment.

Therefore, additional studies should be performed to test their relative efficacy in modifying heat sensitivity on tumor and normal cells.

The clinical results obtained with hyperthermia have been disappointing. This is due to the fact that the original rationale for using hyperthermia in the treatment of cancer was completely reversed by experimental radiobiologists. For example, the original concept of Coley was based on the fact that some tumor cells in humans were very sensitive to heat at temperatures that are commonly reached during viral or bacterial infection. The range of such temperature can vary up to 104°F. The continued use of hyperthermia at low temperatures (103–104°F) as a first mode of treatment would have continued to yield varying degrees of tumor regression without any toxicity. The researchers using mammalian tumor cells in culture failed to obtain any significant increase in cell killing until around 42°C. Therefore, the currently clinically used hyperthermic temperatures, 42–43°C are primarily based on cell culture data. Since these high temperatures can be induced systemically, hyperthermia alone or in combination with radiation can be used only for a local control of a tumor. Hyperthermia is currently used as a last method of treatment when the surviving cancer cells, following chemotherapy and radiation therapy, become highly resistant to these agents. To kill these cells, even high temperatures such as those used in the treatment are not sufficient. The clinical results with hyperthermia may be more encouraging if a systemically induced hyperthermia at 102–104°F is used as a first method of treatment followed by chemotherapy and/or radiation therapy.

1. Inhibitors of Anaerobic Glycolysis

a. 5-Thio-D-glucose (5-TDG)

In 1975, it was reported that 5-thio-D-glucose (5-TDG), an analog of D-glucose, is cytotoxic to tumor cells in culture.¹¹² Subsequently, it was shown^{113,114} that 5-TDG preferentially kills hypoxic tumor cells growing in a monolayer culture or as a multicell spheroid; 5-TDG also potentiates the killing effect of hyperthermia on hypoxic cells *in vitro*.^{115,116} 5-TDG sensitizes the effect of X-irradiation on hypoxic cells, and provides a small degree of radiation protection to oxic cells in culture.¹¹⁷ It also inhibits the uptake of glucose¹¹⁶ and inhibits anaerobic glycosis.¹¹⁹ Because the hypoxic cells depend upon anaerobic glycosis for energy, it is not surprising that 5-TDG is lethal to hypoxic tumor cells. These *in vitro* studies on the effects of 5-TDG on hypoxic tumor cells are very exciting. The fact that 5-TDG is relatively nontoxic in animals suggests that this compound may be potentially more useful in radiation therapy than other hypoxic cell radiosensitizers. The LD₅₀ at 1 day is about 5.5 g/kg in mice.¹¹⁸ Death of the mice always occurred within several hours after injection of the drug. No deaths occurred at doses of 2 to 4 g/kg of body weight; however, the animals showed signs of reduced mobility and disorientation, which disappeared within 2–3 hr.¹¹⁸ It also caused sterility.¹²⁰

Preliminary data suggest that 5-TDG sensitizes the effect of irradiation on mouse Lewis lung carcinoma, but it is only marginally effective against pancreatic duct carcinoma.¹²¹ Preliminary data also show that the continued treatment of mice mammary carcinoma with 5-TDG, mild hyperthermia (41°C), and X-irradiation was more effective in causing regression of tumor than the individual agents. These data indicate that 5-TDG may be a useful radiosensitizer and should be considered for a clinical trial.

b. Butyric Acid

This is a four-carbon fatty acid that occurs naturally in the body and is formed by the hydrolysis of ethyl butyrate. High fiber diet can generate a millimolar level of butyric acid in the colon. Recent studies show that this fatty acid affects morphology, growth rate, and

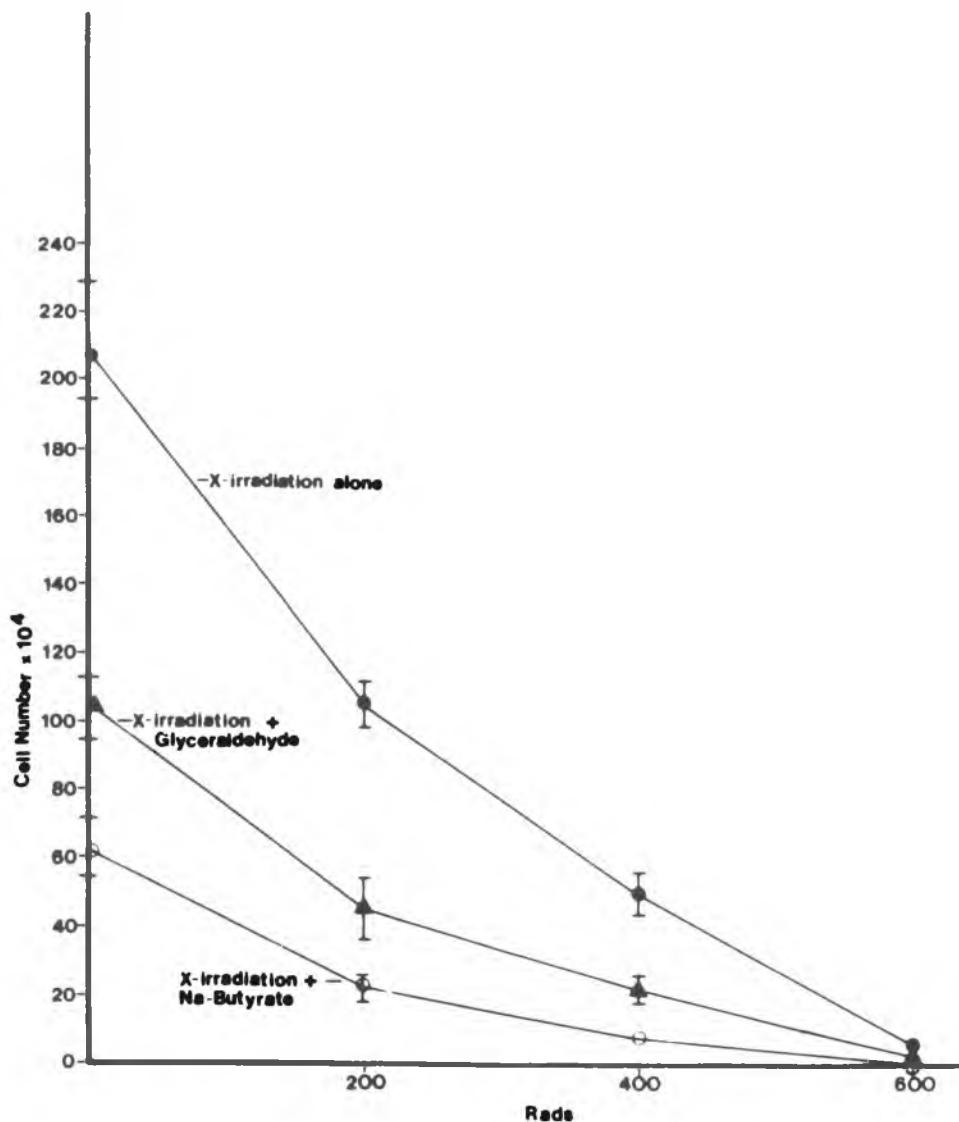


FIGURE 5.6. Effect of inhibitors of anaerobic glycolysis on the growth of mouse neuroblastoma cells in culture. Neuroblastoma cells (50,000) were plated in Falcon plastic culture dishes (60 mm), and sodium butyrate (0.5 mM) and DL-glyceraldehyde (0.25 mM) were added immediately after various doses of X-irradiation. Each value represents an average of at least six samples. The bar at each point is the standard deviation. The vertical bars of the point not shown in the figure were too small to be located on the graph. (From Prasad, K.N., in *Advances in Neuroblastoma Research*, Evans, A.E., Ed., Raven Press, New York, 1980, 140. With permission.)

gene expression in several mammalian tumor cells in culture.^{122,123} Sodium butyrate enhances the growth inhibitory effect of X-irradiation on mouse neuroblastoma cells in culture (Figure 5.6). Because sodium butyrate inhibits anaerobic glycolysis by reducing lactic acid dehydrogenase activity,¹²⁴ and because neuroblastoma cells are more sensitive to inhibitors of anaerobic glycolysis than other cell types,¹²⁵ sodium butyrate-induced cell death and enhancement of radiation response may, in part, be related to inhibition of anaerobic glycolysis. The fact that DL-glyceraldehyde, an inhibitor of anaerobic glycolysis, also

inhibits growth and increases the radiation response of neuroblastoma cells in culture (Figure 5.4), supports the above view.

Sodium butyrate is very nontoxic in humans. Doses of 7–10 g/day produced no clinically detectable toxic effect in patients with neuroblastomas.¹²⁶ Therefore, the author believes that further *in vitro* and *in vivo* studies should be performed to evaluate the role of inhibitors of anaerobic glycolysis. Both 5-thio-D-glucose and butyric acid in combination radiation can be considered for a trial in human tumors. It has already been established that these agents are relatively nontoxic in comparison to previously used radiosensitizers. Studies on the modifications of the effect of radiation injuries are important, because they could enhance our knowledge of radiation injuries and could improve the effectiveness of radiation therapy. Therefore, continued efforts in this particular area must be made.

N. EPIDERMAL GROWTH FACTOR (EGF)

EGF present after γ -irradiation increased the radiosensitivity of human squamous carcinoma cells (A431 cells derived from vulva); however, the radiation response of silta cells derived for carcinoma of the cervix was unaffected by such treatments.¹⁵⁷ The radiosensitization effect of EGF was dependent upon the cell cycle, being highest in the G₁ phase. It was reflected in a reduction of the shoulder of the cell survival curve with almost no change in the slope (Figure 5.7). It is unknown whether EGF will increase the radiosensitivity of normal cells in culture.

O. PENTOXIFYLLINE AND NICOTINAMIDE

Pentoxifylline, a derivative of methylxanthine, has been used in the treatment of some vascular disorders. It increases the disassociation of oxygen from the red blood cells and enhances radiation damage of tumor cells.¹⁵⁸ Nicotinamide, the amide form of nicotinic acid (B₃ vitamins), enhances radiation damage of tumor cells by improving oxygenation.¹⁵⁹ The combination of pentoxifylline and nicotinamide increases radiosensitivity of murine fibrosarcoma by improving the oxygenation of the hypoxic tumor cells.¹⁶⁰

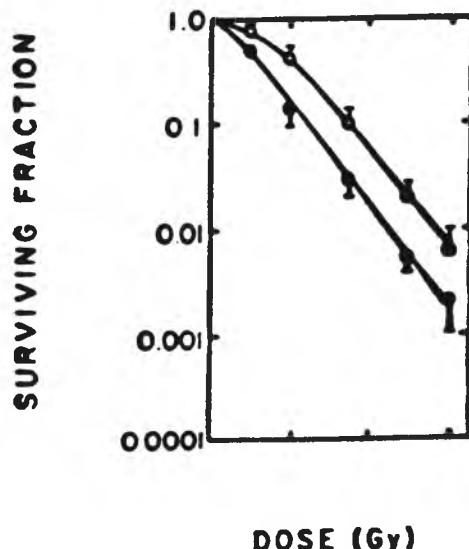


FIGURE 5.7. Radiation dose–response curve of A431 cells in the absence (open symbols) and the presence (closed symbols) of 10 ng/ml EGF during the time of clonogenic assay.¹⁵⁷ (From Kwok, T.M. et al., in *Int. J. Radiat. Oncol. Biol. Phys.*, 20, 525, 1991. With permission.)

P. TAXOL®

Taxol® (paclitaxel) is a plant product derived from the needles and bark of the western yew tree, *Taxus brevifolia*. It exhibits antitumor activity and sensitizes the effect of radiation on the HL-60 human leukemic cell line, astrocytoma, and ovarian cancer cells *in vitro*.^{161,162} The radiosensitizing effect of Taxol® appears to be related to its effect on prolongation of the G₂/M phase of the cell cycle, which is considered the most sensitive phase of the cell cycle.¹⁶⁴ However, a recent study shows that Taxol® sensitizes the effect of Co⁶⁰ γ-radiation on ovarian cancer cells *in vitro* at a dose which is not toxic and which does not cause cell cycle perturbation.¹⁶⁵

Q. RAZOXANE

Razoxane [\pm 1,2-di(dioxopiperazin-1-yl) propane: ICRF159; NSC129, 943] is an anticancer agent and increases the radiosensitivity of some cancer cells *in vitro* and *in vivo*.¹⁶³ The mechanisms of action involve blocking cells in the G₂/M phase of the cell cycle and improving oxygenation by normalizing tumor neovascular development.¹⁶⁶

IV. MODIFYING AGENTS AND HIGH LET RADIATION

All modifying agents such as oxygen, radiosensitizers, and radioprotectors are much less effective in combination with high LET radiation than with low LET radiation.

V. SUMMARY AND COMMENTS

The effect of ionizing radiation on survival can be modified by several physical and chemical agents. Among physical agents, high LET radiation such as α particles and protons are more effective than low LET radiations such as X- or γ-rays. Also, the higher the dose rate, the greater the damage. The effect of a very high dose rate of low LET radiation is equivalent to a low dose rate of high LET radiation. Increased amounts of oxygen increase the radiosensitivity of cells. The oxygen effect is very minimal with high LET radiation. There are several chemicals that provide radiation protection. Among these, sulfhydryl compounds have been most extensively used. These agents are very good radioprotectors when given before X-irradiation, but they are very toxic in humans and do not provide any differential protection of normal and tumor tissues. The WR-2721 compound appears to protect normal tissue preferentially, but its toxicity in humans limits its usefulness. Recent studies suggest that certain physiological substances such as adenosine 3',5'-cyclic-monophosphate (cAMP) and some vitamins (A, C, and E) may be of radioprotective value for normal cells but not for cancer cells. They should be considered for trials in human cancer. Although calcium antagonists are considered great radioprotective agents with minimal toxicity, the specificity of its effect for normal or cancer cells is unknown. There are several chemicals that increase the radiation response of cells; they are referred to as radiosensitizers. Although the analogs of purine and pyrimidine are excellent radiosensitizers, they are very toxic in humans, and they sensitize the effect of irradiation on both normal and tumor cells. Certain hypoxic cell sensitizers, such as electronaffinic compounds (misonidazole), hyperthermia, 5-thio-D-glucose, and butyric acid, have been identified. The clinical results of misonidazole have been very disappointing because of its neurotoxic effects. The clinical results of hyperthermia have been equally disappointing, although it has proven to be very useful in controlling the local tumor problem. Several hyperthermic sensitizers and protectors have been identified. Among them, cAMP, 5-thio-D-glucose, α-tocopheryl succinate, and retinoid are promising.

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Chapter 6

REPAIR OF RADIATION DAMAGE

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I. INTRODUCTION

X-irradiation of the cells simultaneously sets in motion the processes of repair and damage. Therefore, whether the cells live or die depends upon the relative dominance of the repair or damage process. Although it had been known for a long time that irradiated cells repair

damage, Elkind and Sutton-Gilbert¹ were the first to quantitatively define the repair processes in mammalian cells.

Cellular radiation damage can be divided into three categories: (1) sublethal damage, (2) potential lethal damage, and (3) lethal damage. Damage that can be completely repaired by the cells, provided a sufficient time interval after exposure is allowed, is called sublethal damage. In the case of potential lethal damage, the cells can repair radiation injury provided that an appropriate treatment is given after irradiation. Lethal damage of cells can never be repaired. All irradiated cells qualitatively sustain the same damage; the difference between sublethal, potentially lethal, and lethal damage is one of quantity. It is now established that, like bacteria, mammalian cells *in vivo* and *in vitro* also repair radiation damage.

Mammalian survival curves based on the proliferating ability of irradiated cells have been used to interpret the repair processes after irradiation. Survival measurements alone do not reflect the degree of recovery or residual cellular damage. Therefore, the survival measurements indicate only the qualitative process of repair on one criterion.

There are two major purposes for the study of repair processes in irradiated cells: (1) an understanding of the repair process in a cell may permit us to learn something about the process of radiation damage, because the mechanism of repair may be related to the mechanism of damage in a reverse sense; and (2) the study of the repair process gives a basis for the understanding of a similar process *in vivo*, where several intracellular factors are involved.

II. REPAIR OF SUBLETHAL DAMAGE

By using a fractionated-dose or split-dose technique and by measuring the survival of irradiated cells, Elkind and Sutton-Gilbert¹ demonstrated that asynchronous Chinese hamster cells repair sublethal radiation damage (Figure 6.1).

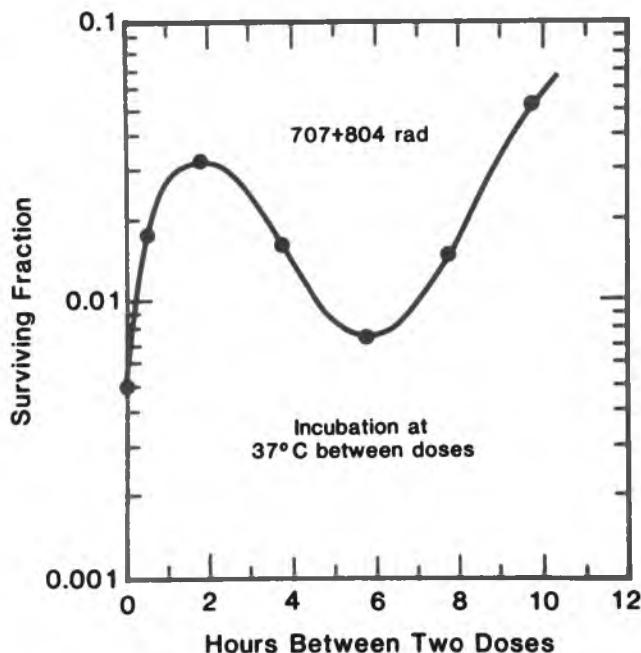


FIGURE 6.1. Survival of Chinese hamster cells exposed to two fractions of X-rays and incubated at 37°C for various time intervals between the two exposures. (From Elkind, M.M., Sutton-Gilbert, H., Moses, W.B., Alescio, T., and Swain, R.W., *Radiat. Res.*, 25, 359, 1965. With permission.)

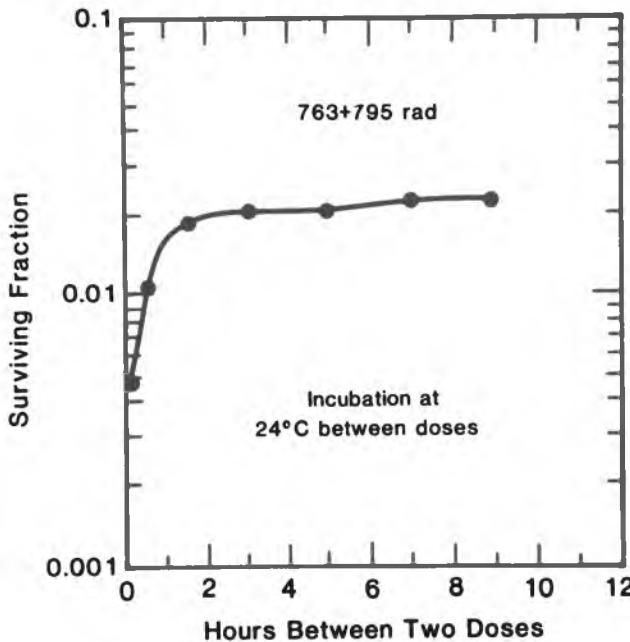


FIGURE 6.2. Survival of Chinese hamster cells exposed to two fractions of X-rays and incubated at room temperature for various time intervals between the two exposures. (From Elkind, M.M., Sutton-Gilbert, H., Moses, W.B., Alescio, T., and Swain, R.W., *Radiat. Res.*, 25, 359, 1965. With permission.)

Significant repair was evident within 3 hr; however, at longer time intervals between two fractions, the surviving fraction decreases, and then increases again to a maximal level.² The decrease in the surviving fraction may be explained as follows. Some of the irradiated cells that survived became arrested in certain phases of the cell cycle (G_2 phase for the Chinese hamster ovary cell). After certain time intervals, these arrested cells resume their movements through the cell cycle. If the second dose of radiation is given at a time when cells are in the most sensitive phase of the cell cycle (e.g., M), the surviving fraction would decrease. To ascertain the above possibility, cells were incubated at 24°C after the first radiation dose. There was no significant movement of cells through the cell cycle at this temperature. Indeed, it was observed that the recovery was complete within 2 hr (Figure 6.2), and no decrease in the surviving fraction was observed under the above experimental condition.

The repair of sublethal damage has been demonstrated in varieties of experimental systems, *in vitro* and *in vivo*. This has also been reviewed in several books and monographs.^{3,4} In most cases, the width of the shoulder of a survival curve reflects the extent of accumulation of sublethal damage.³ The width of the shoulder and the time interval needed to repair the sublethal damage depend upon the radiation factors, growth conditions, and type of cells.

A. EFFECT OF HIGH LET RADIATION

It has long been assumed that the high LET radiation-induced damage is not repairable, whereas the low LET radiation-induced damage is. However, the survival curves after neutron irradiation show significant shoulders, which implies the accumulation of sublethal damage and hence the repair of sublethal damage.⁵⁻⁷ It has recently been reported^{8,9} that the appearance of an exponential survival curve cannot be taken as an indicator of absence of repair. The combined effect of X-irradiation and high LET neon radiation is additive if the neon exposure is preceded by X-rays, and synergistic if the X-rays are preceded by the neon.⁹ It was further noted⁹ that the synergistic effect decreased as cells were allowed time to repair damage. This indicated⁹ that the damage caused by neon or X-rays is repairable. It was concluded,⁹

therefore, that high LET radiation can produce repairable damage in mammalian cells, even though the single-dose survival curve is exponential and the repair of radiation damage is not evident by the dose-fractionation technique.

B. EFFECT OF INHIBITION OF DNA SYNTHESIS

Excess of thymidine (3 mM) completely inhibits DNA synthesis in Chinese hamster cells. This concentration of thymidine had no effect on the repair of sublethal damage, indicating that DNA synthesis is not necessary for the repair process.¹⁰

C. EFFECT OF INHIBITION OF PROTEIN SYNTHESIS

A concentration of puromycin (1–6 µg/ml), which inhibits protein synthesis by about 60% of the controls, does not affect the repair process. One can argue that the remainder of protein synthesis is enough for the repair process. Because of high toxicity, it is not feasible to use a puromycin concentration that would inhibit protein synthesis 100%. Nevertheless, some authors¹⁰ have concluded that the repair of sublethal damage is independent of protein synthesis.

D. EFFECT OF INHIBITION OF RNA SYNTHESIS

Actinomycin D (an inhibitor of RNA synthesis) is capable of suppressing the repair process.¹⁰ A concentration of actinomycin D (0.005 µg/ml), which inhibits RNA synthesis by about 50% of the controls, suppresses the repair process; however, puromycin (6 µg/ml), which inhibits RNA synthesis to the same extent as actinomycin D, has no effect on the repair process. This indicates that no simple relationship between RNA synthesis and the repair process exists.

E. EFFECT OF TEMPERATURE

Cell progression (growth and division) is strongly dependent on temperature, whereas repair of sublethal damage is not. At room temperature (25°C), cell division before or after irradiation is completely suppressed, while the repair of sublethal damage proceeds at a rate compared to that observed at 37°C;³ however, at a low temperature (5°C), the recovery of irradiated HeLa cells is markedly reduced.⁶ In another study, repair of sublethal damage was completely inhibited when HeLa cells were incubated at 20–25°C.¹¹

F. EFFECT OF OXYGEN

Most of the studies indicate that the hypoxic conditions between fractionated doses partially or completely block the repair of sublethal damage.^{12–14}

G. REPAIR OF SUBLETHAL DAMAGE AND CELL CYCLE

Recovery of sublethal damage occurs in all phases of the cell cycle; however, there is some quantitative differences in the rates of recovery.¹⁵ The more resistant phases exhibit a greater capacity for the repair of sublethal damage.¹¹ In all phases of the cycle, there is an initial rise in the rate of repair and then a slow or rapid decline. For Chinese hamster cells irradiated at the end of the G₁ phase, there appears to be an initial rapid restoration, then a plateau, and then a period of rapid recovery. In HeLa cells, significant repair occurred only during the early and mid G₁ phase and in the late S phase.¹¹

H. REPAIR OF SUBLETHAL DAMAGE IN PLATEAU PHASE

Normal human liver (Chang) cells irradiated (500 R + 350 R) during the plateau phase of growth show repair of sublethal damage.¹⁶ On the other hand, when Chinese hamster cells were irradiated in the plateau phase of growth, the slope of the survival curve was similar to that obtained with an exponentially growing culture, but the cells did not accumulate and

repair sublethal damage.¹² These studies suggest that the repair of sublethal damage in cells irradiated at the stationary phase of growth depends upon the type of cells.

I. REPAIR OF SUBLETHAL DAMAGE AND RADIOPROTECTIVE AGENTS

Exposure of cysteamine-treated (2.5 mM) cells to split dose (1600 R + 18 hr + 1600 R) increases the survival by a factor of 1.5 in comparison to the survival after a single exposure to the same total dose when anoxic conditions prevailed during the irradiation.¹⁷ When oxygenated conditions were maintained during irradiation, exposure to split doses increased the survival of both the cysteamine treated and untreated cells by a factor of about 2 in comparison to a single exposure to the same total dose. It has been suggested that cysteamine prolongs the time period during which the radiation damage remains recoverable before it is irreversibly fixed.¹⁷

J. REPAIR OF SUBLETHAL DAMAGE AND RADIOSENSITIZING AGENTS

5-Bromodeoxyuridine (5-BrdU), an analog of thymidine, enhances the radiation resistance of mammalian cells in culture. It has been shown that 5-BrdU reduces the repair of sublethal damage.¹⁸

K. REPAIR OF SUBLETHAL CHROMOSOME DAMAGE

In addition to repair of sublethal damage based on the criterion of survival, mammalian cells also repair sublethal chromosomal damage.¹⁹ This is shown by the fact that the response of two 300-rad doses of X-rays is additive. A significant amount of repair occurred within 10–20 min, and the repair was essentially complete by 1–2 hr. The half-time for the repair process is about 12 min. The repair did not depend upon protein synthesis, because it occurred in the presence of cycloheximide, which suppresses the uptake of ³H-leucine by 95%. Furthermore, repair was seen for both deletions and exchanges, although there appeared to be more repair based on exchanges than on deletions. No repair was observed in terms of chromosomal aberrations or cell mortality when the cells were held at 0°C for as long as 1 hr between two 300-rad doses. The excellent agreement between the amount of cell killing and the number of chromosomal aberrations, and between the repair of lesions responsible for the cell death and those responsible for chromosomal aberrations — as the cells were irradiated under different conditions — strongly supports the hypothesis that X-ray-induced cell death results from damage in the chromatin structure.¹⁹

Using the premature chromosome condensation technique of Johnson and Rao,²⁰ it was demonstrated that the chromatid breaks and gaps of irradiated Chinese hamster cells (CHO) can be repaired with a half-time of about 1 hr; but exchanges, once formed, cannot be repaired.²¹ Using this technique, it was shown²¹ that only about one-third of the chromatid breaks induced by α -irradiation could be repaired in 1 hr, whereas about one-half the chromatid breaks induced by γ -irradiation were repaired during this time. Thus, the above studies further show that a portion of high LET radiation-induced damage is repairable.

III. REPAIR OF DNA

Both single-strand and double-strand breaks of DNA induced by ionizing radiation are repairable.²² When a 20-min time interval is allowed after irradiation, irradiated cells rapidly repair single-strand breaks. It has been shown that in *Escherichia coli* DNA polymerase is required for the rapid repair of X-ray-induced DNA strand breaks *in vivo*.²³ This is shown by the fact that a much higher yield of DNA single-strand breaks was obtained in the DNA polymerase-deficient mutants, *E. coli* K-12 pol Al, after a given dose of X-rays than had been

found before in *E. coli*. The increased yield of single-strand breaks was due to the absence of a rapid repair system, which had not been described in *E. coli* K-12. The rat thymus lymphocytes are low in both DNA polymerase and ligase, which are enzymes that are required for repair synthesis; however, they are able to effectively carry out the rejoining of their single-strand breaks induced by X-irradiation.²⁴

It has been reported^{25,26} that the repair of DNA exhibits fast and slow components. In an attempt to correlate the damage and repair of DNA with chromosomes, it was observed²¹ that the frequency of chromosomal aberrations remained constant for at least 30 min after irradiation, while more than half of the DNA lesions were repaired within 5 min. Based on this study, it was concluded²¹ that the fast-repairing DNA lesions (probably single-strand breaks and the alkali-labile region) are not important in the formation or repair of chromosomal breaks. However, the more slowly repaired DNA lesions may be responsible for lesions in chromosomes.

A. THE "CUT AND PATCH" HYPOTHESIS OF DNA REPAIR

It has been postulated²⁷ that cultured mammalian cells repair radiation-induced DNA damage by the "cut-and-patch" mechanism. This mechanism of repair has been explained as follows:

1. Radiation interacts with nuclear DNA. This interaction causes alteration of the bond structure of the DNA molecule. For modeling purposes, it was assumed that bond abnormalities occur at the site of interaction of radiation with DNA.
2. Nucleases scan the DNA molecule for radiation-induced bond abnormalities and convert the bond abnormalities into actual breaks.
3. If the break involved only one of the DNA strands, it is closed by the action of the repair enzyme. Thus, the single-strand DNA breaks are considered to be repairable radiation damage.
4. If bond abnormalities occur in both DNA strands, this would result in a double-strand break. The double-strand break is considered lethal.

B. DNA REPAIR IN VIVO

The repair of DNA also occurs *in vivo*, but the rate and extent of repair are different from those observed *in vitro*. For example, it was reported that X-irradiation produced single-strand breaks with equal efficiency in the DNA of differentiated neurons *in vivo*, and CHO cells *in vitro*; however, the rejoining processes of the DNA was different.²⁸ It has been shown²⁹ that the number of breaks was greater when mouse thymocytes and hepatocytes were irradiated *in vitro* than when these cells were irradiated *in vivo*. However, the rejoining was detected in thymocytes irradiated *in vitro* and *in vivo*, but only in hepatocytes irradiated *in vivo*. Furthermore, the nondividing neurons of the rat cerebellar region repaired radiation-induced DNA damage to the same extent as did the dividing rat intracerebellar 9L tumor cells.³⁰ However, the rate of repair in neurons was slower. In general, the DNA damage of cells irradiated *in vitro* is more sensitive to radiation than those irradiated *in vivo*.³¹

C. REPAIR REPLICATION

Repair replication is a term used to describe a kind of DNA synthesis that is not semiconservative in nature, occurs after damage to DNA has occurred, and involves the insertion of nucleotides into extant strands of DNA. In bacteria, there is good evidence that this process is involved in the recovery of cells from damage induced by UV irradiation. An identical process also occurs in mammalian cells.³² The repair replication is important in maintaining the reproductive integrity of human cells after UV irradiation.³³ The situation is far less clear for repair replication occurring after exposure to ionizing radiation. Repair

replication was first reported³⁴ in HeLa cells after high exposures (10,000–100,000 R). Later, repair replication in G₁ HeLa cells was reported to occur after doses as low as 500 R; the majority of “repaired” DNA later replicated normally by the semiconservative mode.³⁵ It has been reported³⁶ that repair replication occurred in P-388F (mouse) cells after doses as low as 150 R. The amount of repair replication occurring in these cells after 150 R was extremely large; for higher doses, the relative amount of repair synthesis was even greater. However, it has been suggested³⁷ that the extensive DNA synthesis reported after the 150-R dose does not represent repair synthesis, because the synthesis is not restricted to damaged sites in DNA. In addition, it has been calculated³⁷ that the degradation at each damaged site does not exceed three bases; this small amount of base insertion cannot be detected in the presence of the nonconservative synthesis occurring in controls until the damage to DNA is extensive, i.e., more than that caused by 1000 rads.

IV. REPAIR OF POTENTIAL LETHAL DAMAGE (PLD)

In addition to repairing sublethal damage, mammalian cells can also repair potential lethal damage. This damage ordinarily causes cell death, but its expression can be prevented by postirradiation treatment. The latter process is also referred to as potentially lethal damage repair (PLDR). The selective inhibitors of PLDR for tumor cells may enhance the efficacy of radiation therapy of cancer.

A. EFFECT OF INHIBITION OF DNA SYNTHESIS

The inhibition of DNA synthesis by excess of thymidine (2.5 mM) or by hydroxyurea for a period of 4 hr enhanced the survival of irradiated HeLa cells.³⁸ But the effect was dependent upon the cell cycle. For example, thymidine was effective when the cells were irradiated in S phase or G₁-transition S phase, whereas hydroxyurea was effective when the cells were irradiated at S phase. The opposite results were obtained by other groups of investigators.^{39,40} Another study⁴¹ showed that inhibitors of DNA synthesis (excess thymidine, hydroxyurea, and cytosine arabinoside) at nontoxic concentrations, when added after irradiated for a period of 4 hr, had no effect on the survival of irradiated CHO cells in culture. Thus, the role of DNA synthesis in repair of potential lethal damage remains to be ascertained.

B. EFFECT OF INHIBITION OF RNA SYNTHESIS

Actinomycin D (0.005 µg/ml), an inhibitor of RNA synthesis, reduces the survival of irradiated Chinese hamster cells when added after irradiation.^{41,42} In addition, acriflavine (0.25 µg/ml), which has the property of binding to nucleic acids, also reduces the survival of irradiated Chinese hamster cells when given immediately after irradiation. Puromycin amino nucleoside, an inhibitor of ribosomal RNA synthesis at a nontoxic level, reduced the survival of irradiated HeLa cells when added after exposure.¹⁶ Thus, it appears that agents which bind to DNA interfere with the repair of potential lethal damage. RNA synthesis appears to be necessary for the irradiated cell to repair potential lethal damage.

C. EFFECT OF INHIBITION OF PROTEIN SYNTHESIS

Puromycin (5 µg/ml), an inhibitor of protein synthesis, decreases the survival of HeLa cells irradiated in the S phase; however, when added after irradiation for a period of 4.5 hr, in cells irradiated in the middle of the G₁ period, the surviving fraction slightly increased.³⁸ When cycloheximide (8 µg/ml) was added to HeLa cells irradiated (450 rads) in mid G₁ for a period of 6 hr, it increased the survival of irradiated cells.³⁹ This agent also protected cells against the radiosensitizing effect of hydroxyurea. In Chinese hamster ovary cells, the inhibition of protein synthesis by cycloheximide (5 µg/ml) and puromycin (5 µg/ml), after irradiation for

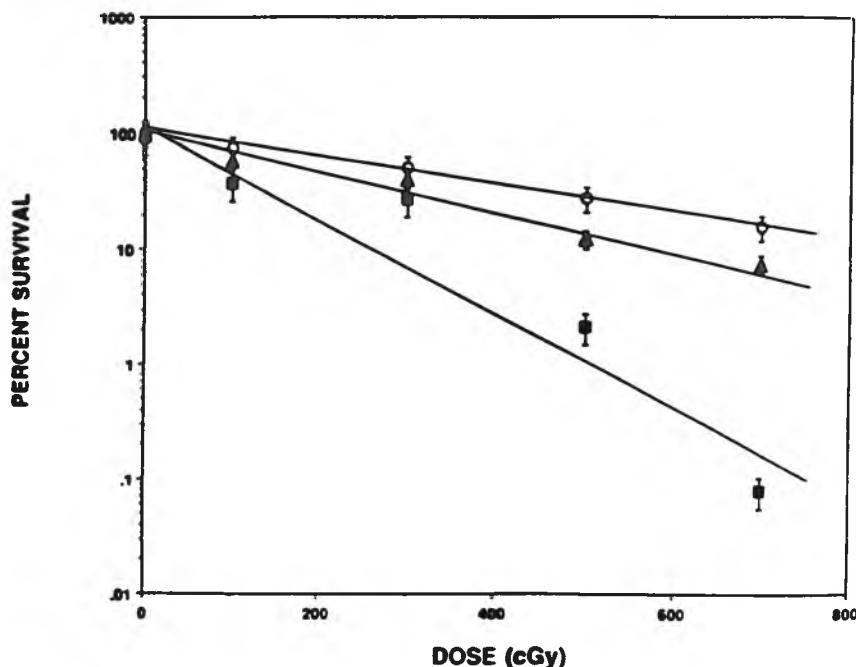


FIGURE 6.3. Ability of an optimal concentration of camptothecin to sensitize human U1-Mel cells at various doses of ionizing radiation. Confluence-arrested U1-Mel cells were untreated or exposed to various doses of ionizing radiation. Immediately after X-irradiation, cells were treated with 4 μ M camptothecin for 4 hr (■) and then replated; immediately trypsinized and replated (▲), and allowed to repair for 4 hr without drug treatment (○).

4 hr, had no effect on the survival of irradiated cells.⁴¹ This finding is in contrast to the finding reported for HeLa cells. Unfortunately, this study was performed with an asynchronous cell population; therefore, this conclusion is questionable.

D. INHIBITORS AND ACTIVATORS OF TOPOISOMERASE

β -Lapachone, an activator of topoisomerase I, enhanced radiation-induced lethality by inhibiting PLDR.⁴⁹ Camptothecin, a specific inhibitor of topoisomerase I, markedly enhanced radiosensitivity of human melanoma (U-Mel) when it was given during or immediately after X-irradiation for 4 hr.⁵⁰ Enhanced cell killing by camptothecin was proportional to the extent of radiation-induced initial damage; the higher the radiation dose, the greater the radiosensitization (Figure 6.3). Camptothecin was ineffective when given before X-irradiation. The clinical relevance of β -lapachone or camptothecin in improving radiation therapy has not been evaluated.

E. POLY(ADENOSINE DIPHOSPHORIBOSE)

The synthesis and activity of poly(adenosine diphosphoribose) are enhanced in mammalian cells following X-irradiation.⁵¹ The extent of increase in enzyme activity was greater in a resistant human squamous cell carcinoma cell line (1483) than that observed in a radiosensitive line (183A). This suggests that poly(adenosine diphosphoribose) plays an important role in the repair of potential lethal damage.

F. EFFECT OF cAMP

The survival of exponentially growing JTC cells, derived from ascites tumor cells, was enhanced when these cells were continuously treated with dibutyryl cAMP for at least 6 hr

before and 3 hr after X-irradiation.⁴³ While the D_q (522 rads) did not change, the D_0 increased from 71 to 101 rads. This was interpreted to mean that dibutyryl cAMP helps in the repair of potential lethal damage, since the postirradiation treatment was found to be necessary.

G. EFFECT OF TEMPERATURE

When HeLa cell cultures were irradiated (450 rads) in early G₁ and incubated for various lengths of time at 29°C, the survival of irradiated cells decreased.³⁹ This was true for cells irradiated in all phases of the cycle. The survival was depressed to the same extent when cells were incubated at 5°C for 4 hr after irradiation. The low temperature (29°C) had no effect on the viability of nonirradiated cells or as a preirradiation treatment. It has been suggested³⁹ that the low temperature probably interferes with the repair process. Some investigators obtained contrasting results using different mammalian cell cultures. The exposure of mouse L cells⁴⁴ and leukemic cells⁴⁵ to suboptimal temperature (29°C) after irradiation increases the survival of irradiated cells. Other workers have confirmed the above finding.

When Chinese hamster cells were irradiated at 20°C with a single dose (600 rads) and then incubated at 20°C for various periods of time, potential lethal lesions and potential chromosomal aberrations were repaired. At 20°C the metabolism of cells was greatly reduced, and the incorporation of ³H-leucine was inhibited by 95%, which suggest that a process responsible for confirming damage was also inhibited or suppressed, while the repair process was affected very little.¹⁴ The repair kinetics for the lesions with the potential for forming chromosomal aberrations were almost identical to the repair kinetics for the lesions with the potential for cell death, and very similar to the kinetics for repair of sublethal damage. These positive correlations suggest that potential lethal damages are derived from interactions of sublethal damage. Furthermore, these potential lethal lesions, if they are not repaired, appear to be responsible for both chromosomal aberration and cell death.¹⁹ There was repair of potential lethal damage when Chinese hamster cells were incubated at 20°C after irradiation (600 rads) for as long as 1 hr. Thus, it seems the suboptimal temperature (20°C) promotes the repair of potential lethal damage both at the level of survival and of chromosomal damage, whereas very low temperature (0°C) inhibits both sublethal and potentially lethal damage.

H. EFFECT OF CONDITIONED MEDIUM FROM STATIONARY CULTURES

When Chang human liver cell cultures were allowed to reach the stationary phase of the plateau phase of growth, the proliferative activity of the cells was markedly reduced. When such a culture is X-irradiated (400 to 1000 R), cellular survival may be considerably enhanced if, before subculturing the cells to assay for colony-forming ability, the culture is left in the stationary phase for 6 hr.⁴⁶ This effect may be due to the presence of a diffusible substance in the culture medium that probably promotes repair of potentially lethal damage in either stationary or exponentially growing cells. The release of this substance by stationary cells is not independent of irradiation. This substance is sensitive to heat (50°C) but is insensitive to treatment with DNase and RNase.

Chinese hamster cells can repair potentially lethal radiation damage if incubated in Earle's balanced salt solution immediately after a single exposure.⁵ Not only the parent cells, but the daughter cells as well, retain the capacity to repair potentially lethal damage.⁴⁷ This is demonstrated in the following experiment. Lung cells of the female Chinese hamster (V79-4) were X-irradiated (800 R) and incubated at 37°C. When the average number of cells per colony (N) of irradiated cells was two to three — usually within 20–24 hr after irradiation — the medium was removed, cells were rinsed with Puck's saline F(PSF), and the rinse solution was discarded and replaced with a fresh buffer. After incubation in the buffer for various intervals of time, the buffer was removed and a fresh growth medium was added for colony formation.

V. RECOVERY OF SUPRALETHALLY X-IRRADIATED AND NITROGEN MUSTARD-TREATED CELLS

Large, multinucleated amoebae, *Pelmyxa illinoiensis*, irradiated with 3000 R of X-rays (supralethal dose), died after 4–5 days. Amoebae treated with a supralethal concentration (0.1–0.2 mg/ml for 1 hr) of nitrogen mustard (HN_2) died within 12 days after treatment without cell division. When protoplasm (cytoplasm plus nucleus) from an HN_2 -treated amoeba was injected by fusion into an X-irradiated (30,000 R) amoeba, the composite organisms reproduced and permanently recovered. HN_2 probably alkylates the donor amoeba DNA without affecting the cytoplasmic factors. Therefore, cytoplasm from HN_2 -treated donors also induced recovery.⁴⁸ The irradiated amoebae also showed complete recovery when they received protoplasm from nonirradiated amoebae within 24 hr after exposure. The cytoplasm from nonirradiated amoebae also prevented radiation death.

HN_2 -treated amoebae, unlike X-irradiated amoebae, did not recover after the injection of cytoplasm from nontreated, nonirradiated amoebae. Actinomycin D (50 µg/ml, for 24 hr), an inhibitor of messenger RNA as well as transfer and ribosomal RNA synthesis, breaks polyribosomes. Actinomycin D-treated donor amoeba protoplasm failed to restore X-irradiated recipients.⁴⁸ Puromycin dihydrochloride (0.1–0.3 mg/ml, for 24 hr) inhibits amino acid incorporation into protein by attaching to the growing ends of nascent polypeptide chains and by reducing the size of polyribosomes. Puromycin-treated donor protoplasm prevented the recovery of irradiated amoebae; these results indicate that protein synthesis is required by amoebae for recovery from radiation injury. Tetracycline or oxytetracycline inhibits protein synthesis by interfering with the binding reactions of transfer RNA to the ribosome messenger RNA complex, and tends to localize in the mitochondria. 2,4-Dinitrophenol, an uncoupler of oxidative phosphorylation, secondarily interferes with protein and nucleic acid synthesis. When a donor amoeba was treated with any of the above agents, it failed to restore the reproductive capacity of supralethally irradiated amoebae.⁴⁸ Some authors have postulated that polyribosomes and their larger subunits from nontreated (or HN_2 -treated) amoebae produce recovery of X-irradiated amoebae.⁴⁸

VI. SUMMARY AND COMMENTS

All irradiated cells qualitatively sustain the same damage; the difference between sublethal, potential lethal, and lethal damage is one of quantity. All mammalian cells in culture repair sublethal damage, provided enough time is allowed between two doses. The repair of sublethal damage is independent of protein and DNA synthesis. Actinomycin D, an inhibitor of RNA synthesis, interferes with the repair of sublethal damage; however, puromycin, which inhibits protein and RNA synthesis (to the same extent as actinomycin D), had no effect on the repair process. Thus, there appears to be no simple relationship between RNA synthesis and the repair of sublethal damage. Although a suboptimal temperature (25°C) had no effect on the repair process, a low temperature (5°C) markedly reduced the repair of sublethal damage. Most of the studies agree that the hypoxic condition of cells after irradiation interferes with the repair process. When cells were irradiated with high LET radiation (survival curve without a shoulder), some recovery did occur. A low dose rate produced a greater number of survivors than did a high dose rate. Although the recovery of sublethal damage occurs in all phases of the cell cycle, it is most pronounced in the S phase, which is also the most radioresistant phase of the cycle. The cells do not repair when irradiated in the plateau phase of growth. Radioprotective agents probably prolong the time during which the radiation damage remains recoverable before it is fixed irreversibly. On the other hand, radiosensitizing agents reduce the capacity of cells to accumulate sublethal damage; therefore, less repair occurs.

Repair of sublethal damage also occurs at the chromosomal level. Single-strand breaks of DNA rejoin within 20 min of exposure. The repair of DNA occurs because of the presence of repair enzymes. Repair replication occurs after damage to DNA and involves the insertion of nucleotides, presumably after excision of damaged nucleotides into the extant strand of DNA. This type of repair is observed after a supralethal dose of irradiation.

All mammalian cells repair potential lethal damage provided an appropriate treatment is given after irradiation. One group of investigators reported that the inhibition of DNA synthesis, and not the inhibition of protein synthesis, allows the repair of potential lethal damage. Others have suggested that the reverse is true. The inhibition of RNA synthesis reduces the repair process. A suboptimal temperature (25°C) allows the repair of potential lethal damage, whereas a low temperature (2°C) does not allow any recovery. The conditioned medium obtained from stationary cultures facilitates the repair process when irradiated cells are allowed to remain in the medium for at least 6 hr. β -Lapachone, an activator of topoisomerase I, and camptothecin, an inhibitor of topoisomerase I, markedly enhanced radiosensitivity of tumor cells in culture by inhibiting the potential lethal damage repair (PLDR) process. Poly(adenosine diphosphoribose) appears to play an important role in the repair of potential lethal damage.

Some unicellular organisms can repair radiation damage after a supralethal dose. Supralethally X-irradiated amoebae reproduced and permanently recovered, provided that they received nonirradiated cytoplasm or nitrogen mustard-treated cytoplasm. Actinomycin D-, puromycin-, or dinitrophenol-treated donor cytoplasms, failed to restore the X-irradiated amoebae, indicating that both RNA and protein synthesis are necessary for the repair of radiation damage after a supralethal dose.

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Chapter 7

MOLECULAR RADIATION BIOLOGY

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L INTRODUCTION

To understand the mechanism of radiation, it is necessary to understand radiation effects on the molecular level. Since deoxyribonucleic acid (DNA) is considered the key molecule for the regulation of growth and differentiation, the effects of ionizing radiation on the structure, function, and synthesis of DNA are first examined. Recent identifications of cellular oncogenes and genes have provided new opportunities to investigate the effects of ionizing radiation on gene expression. In addition, the role of oncogenes in radiosensitivity is also being investigated.

II. EFFECT OF RADIATION ON DNA

The effect of radiation on DNA is dependent on dose, time, and the phase of the cell cycle.

A. DAMAGE OF DNA IRRADIATED *IN VITRO*

X-irradiation (more than 1000 rads) of DNA solution *in vitro* produces the following types of damage: (1) breakage of hydrogen bonds; (2) chain breaks, (3) cross-linkage, (4) disruption of the sugar-phosphate backbone of DNA, (5) impairment of the transforming ability of DNA, (6) DNA base damage, and (7) inability of the DNA to act as a template for the synthesis of a new DNA strand.

B. RADIOSENSITIVITY OF SINGLE-STRANDED VS. DOUBLE-STRANDED DNA

The single-stranded DNA is a more effective template for the synthesis of new DNA than the double-stranded one; therefore, the relative radiosensitivity of single- and double-stranded DNA has been investigated. When DNA is irradiated *in vitro*, single-stranded DNA is more radiosensitive than double-stranded, on the criterion of base damage.⁵²

C. DAMAGE OF DNA IN IRRADIATED CELLS

Several properties of DNA (DNA content/cell, deoxyribose level, viscosity, sedimentation, and priming ability) do not show any significant change when DNA is isolated immediately after irradiation of cells (750–1000 R); however, when more time is allowed, some changes of DNA can also be demonstrated after supralethal exposure.²

In double-stranded viruses, one double-strand cut of the DNA molecule is considered lethal; however, in mammalian cells, multiple single-stranded breaks and double-stranded cuts are required to kill a cell. DNA molecules of cultured mammalian cells are 10,000 to 1 million times longer than those of viral DNA and exist in close association with histones and other chromosomal proteins.

D. DAMAGE OF DNA SYNTHESIS

The initiation of DNA synthesis in the slowly dividing human amnion cells in culture is much more radiosensitive than that in the rapidly proliferating cells.²⁸ In HeLa⁴⁷ and Chinese hamster ovary cells,⁴⁶ DNA synthesis rate is maximally depressed (about 50%) when cells are X-irradiated (500–750 R) in the S phase. These exposures also prolong the total DNA synthesis period. When Chinese hamster ovary cells are irradiated in the G₁ or G₂ phase, DNA synthesis is essentially unaffected in the subsequent cycle, except for the division delay. However, HeLa cells do not behave very differently than CHO cells. When HeLa cells are irradiated during the G₁ phase, the rate of thymidine incorporation is reduced in a dose-dependent fashion, so that about two-thirds of the normal amount is incorporated after

500 rads. It has been shown that virtually the entire cell population completes the first cell-cycle after irradiation with 500 rads, but that about 25% of cells are permanently arrested in mitosis at the end of cell-cycle. Those cells that are going to be arrested in mitosis synthesize DNA at only 30% of the normal level. The cells divide at about 80% of the normal amount. This severe deficiency in DNA synthesis is closely related to permanent mitotic arrest.¹⁸

Lajtha et al.,²⁷ in human bone marrow cell culture, have shown that the dose-response curve for the rate of incorporation of radioactive precursor into DNA during 1–4 hr of the postirradiation period is biphasic, i.e., the curve has two components. Such biphasic curves are also obtained by irradiating HeLa cells, regenerating rat liver, and several other cell lines. The dose-effect curve of DNA synthesis is also biphasic after irradiation of HeLa cell nuclei or cytoplasms with an α particle microbeam.²⁶ Irradiation of up to half of the cytoplasm also results in a significant (up to 18%) inhibition of DNA synthesis 1 hr later, but the dose to the cytoplasm needs to be 10 times larger than the dose to the nucleus. Many hypotheses have been advanced to explain the biphasic nature of the dose-response curve. Some of them include (1) the fast (radiosensitive, D_0 for human bone marrow culture = 500 rads) component results from the inhibition of nuclear phosphorylation, and the slow (radioresistant, D_0 for human bone marrow culture = 13,000 rads) component results from damage to the DNA template; (2) the fast component is a consequence of damage to the DNA template; (3) the fast component results from competitive inhibition of exogenous labeled thymidine incorporation due to the presence of excess DNA breakdown products in a cell; and (4) the fast component is attributed to the disorganization of deoxyribonucleoprotein structures.

An essential aspect of the radiobiologic findings with the regenerating mammalian liver system is that small doses of radiation, given before the onset of DNA synthesis, delay synthesis. Once DNA synthesis has begun, higher doses are required to yield an equivalent degree of inhibition of synthesis. An exposure of 1500 R to the liver during any period of regeneration completely inhibits DNA synthesis 24 hr after irradiation.⁶

E. DOSE-TIME RELATIONSHIP OF ALTERED DNA SYNTHESIS

There are many studies of the time course of altered DNA synthesis and of DNA content in irradiated asynchronous cell populations. Changes in the DNA synthesis and DNA content reflect not only what is happening in irradiated S phase cells, but also the inflow and outflow of cells, and the formation of giant cells. Therefore, it is necessary to exclude these variables while discussing the dose-time relationship of DNA synthesis in the S phase. In general, irradiation causes depression of the DNA synthesis almost immediately and reaches a minimum value within 1 hr, but revives in 2–5 hr after exposure. Upon resumption of the DNA synthesis in mouse leukemic cells, the rate of DNA synthesis is 20% higher than that of nonirradiated S phase cells. This excess of DNA synthesis may be attributed to "repair synthesis."⁴⁵ The repair synthesis differs from the "normal" DNA synthesis of S phase cells³² in at least the following three respects: (1) repair synthesis occurs generally in all phases of the cell cycle and is often called "unscheduled DNA synthesis," (2) repair synthesis occurs in parental as well as daughter strands (replicating strands) of DNA, and (3) repair synthesis is not inhibited by hydroxyurea, an inhibitor of normal DNA synthesis.

F. MECHANISMS OF DEPRESSION OF DNA SYNTHESIS

The exact mechanism of radiation-induced depression of DNA synthesis is unknown. Many hypotheses have been suggested, some of which include the following: (1) the DNA polymerase activity decreases, and (2) the pool size of DNA precursors may be altered either by a change in membrane permeability or by an alteration of other biochemical factors necessary for the synthesis of nucleosides and nucleotides.

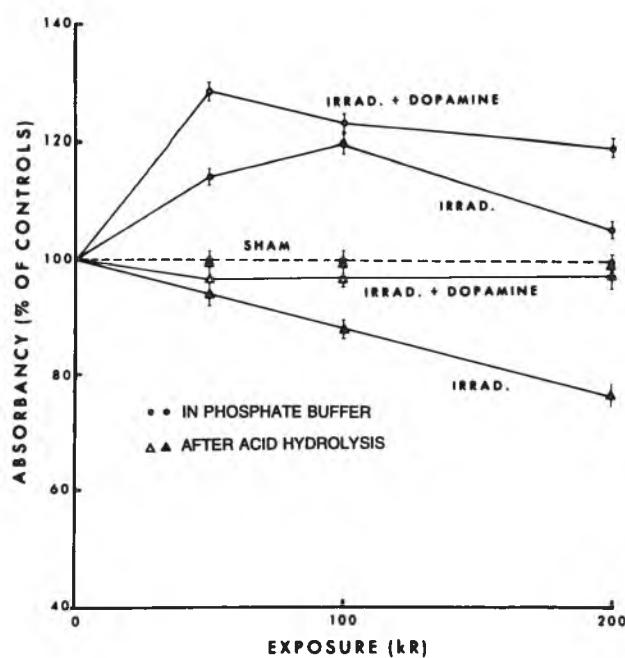


FIGURE 7.1. Effect of dopamine (31 µg/ml) on DNA X-irradiation *in vitro*, the dopamine-DNA ratio being 1:13. Absorbancy at 260 nm of irradiated DNA solution in phosphate buffer and in 0.1 N HClO₄, after acid hydrolysis was determined. Each point represents an average of at least four samples. Vertical bars represent standard errors of the mean (95% confidence interval). ○-● = in phosphate buffer; △-▲ = after acid hydrolysis. DNA solution irradiated in the presence of dopamine shows no change in absorbancy when compared to controls. (From Prasad, K.N., *Int. J. Radiat. Biol.*, 14, 79, 1968. With permission.)

G. MODIFICATION OF DAMAGE OF DNA SYNTHESIS

The presence of oxygen increases twofold the frequency of radiation-induced single-strand breaks in mouse leukemic cells. Dopamine³⁴ reduced DNA damage (chain breaks and base damage) induced by X-ray exposure of 100,000 R (Figure 7.1). Catecholamines scavenge free radicals *in vitro*; therefore, it is not surprising that dopamine protects DNA against some types of radiation damage. Certain SH compounds also protect DNA against radiation damage when present during irradiation of DNA *in vitro*.²⁴

H. "UNSCHEDULED" DNA SYNTHESIS IN HUMAN CELLS

DNA synthesis can be induced in all phases of the cell cycle of mammalian cells by such agents as UV rays, X-irradiation, and alkylating agents. This so-called "unscheduled" DNA synthesis is of low magnitude compared with normal DNA synthesis in the S phase, and has been equated to repair activity in damaged cells. Supralethal doses of ionizing radiation (5000–10,000 rads) induce "unscheduled" DNA synthesis in HeLa cells.³² Such an observation has been confirmed in human lymphocytes and other mammalian cells. In addition, a recent report⁴⁹ shows that significant amounts of ³H-thymidine are incorporated into the DNA of HeLa cells without an extracellular stimulus, and at a time when normal DNA synthesis does not occur. It has been suggested that lesions are introduced into the genome as part of a general error-correcting mechanism or during normal replication and transcription potentiated by exonuclease activity. The spontaneous, unscheduled DNA synthesis may account for the repair of such lesions induced as a part of the cell's normal metabolic activity. The induction of unscheduled DNA synthesis in mammalian cells by X-irradiation further suggests that such a metabolic event plays a role in the repair of DNA molecules.

III. EFFECT OF RADIATION ON RNA

There are at least three types of RNA in mammalian cells: messenger (m) RNA, transfer (t) RNA, and ribosomal (r) RNA. Most studies have measured the effect of radiation on total RNA synthesis. Such studies cannot be interpreted in relation to individual RNA species. Because of the complexities involved in RNA structure and function, the effect of radiation on RNA is relatively little understood. In general, it appears that the total RNA synthesis is less radiosensitive than the DNA synthesis. This may be true only within a certain time limit. In Jensen rat sarcoma, an exposure of 2000 R reduces ^{32}P uptake into DNA by as much as 76% when measured 1.5 hr after irradiation; this exposure does not affect the ^{32}P incorporation into RNA. However, when the incorporation of ^{32}P is measured 8 hr after irradiation, the extent of incorporation into DNA and RNA is decreased to about the same level. In rat regenerating liver, an exposure of 1500 R causes marked inhibition of DNA synthesis, but an even higher exposure (3000 R) has no effect on RNA synthesis 24 hr after irradiation.⁶ In rabbit bone marrow, an exposure of 800 R reduces DNA and RNA synthesis by approximately equal amounts during a 5-hr interval after irradiation.¹ In mouse L cells, an exposure of 2000 R has no effect on RNA synthesis for a period of about one generation. After this, the synthesis continues at a much reduced rate for a long period of time.⁵⁵ Many studies have found little or no depression of RNA synthesis for a period of 30 min to 6 hr after irradiation of mammalian cells with less than 2500 rads of X- or γ -rays. Irradiated CHO cells are stimulated to synthesize RNA at a higher rate than the controls.⁴⁴ The effect is more pronounced at the G₁ period.

X-irradiation (600–1500 R) of rat regenerating liver 18 hr after hepatectomy inhibits the incorporation of ^{14}C -orotic acid in rapidly labeled RNA 6 hr after irradiation (Figure 7.2). However, it remains to be established that rapidly labeled RNA manifests properties of messenger RNA, and that the fraction of rapidly labeled RNA whose specific activity is inhibited by X-irradiation contains mRNA. No measurable effect of irradiation on the incorporation of ^{14}C -orotic acid into rapidly labeled RNA of normal rat liver was observed. Other authors have shown an enhanced synthesis of rapidly labeled RNA in bacteria and in some mammalian tissues. An increase in RNA synthesis and RNA polymerase activity in X-irradiated (600 R) rat liver nuclei was also observed. Since the corticoid hormones increase the synthesis of RNA and RNA polymerase, and X-rays are known to stimulate the adrenals, it has been suggested that an increase in RNA synthesis is probably due to the stimulation of the adrenals by X-irradiation.

Local irradiation (15,000 R) to the head and whole-body irradiation (500 R) of a rat result in a time-dependent inhibition of cytoplasmic RNA labeling 24–48 hr after the exposure of a nerve cell.⁵⁷ A dose of 500 R to the total body results in a greater depression of cytoplasmic RNA labeling than does 500 R to the head alone. Labeling of nuclear RNA immediately after irradiation is not sufficiently different in irradiated and nonirradiated animals to account for the subsequent difference in the cytoplasmic labeling.

A. DAMAGE OF RIBOSOMAL RNA SYNTHESIS

The effect of ionizing radiation on RNA synthesis has been studied in HeLa S3 cells grown in suspension culture. In the first hour following 1000 rads, RNA synthesis is depressed 30–50%. The rate of RNA synthesis returns to a normal level 24 hr after irradiation; the RNA synthesis is not inhibited.⁵ In rat thymocyte, X-irradiation (1100 R) within 1 hr inhibits the maturation of rapidly labeled RNA to stable RNA, as shown by the fact that prelabeled RNA radioactivity is lost (31%) within 2 hr of irradiation, whereas radioactivity in prelabeled DNA is not lost. The magnitude of the loss of radioactive label from RNA is related to the time interval between pulse labeling and subsequent X-ray exposure.¹⁴

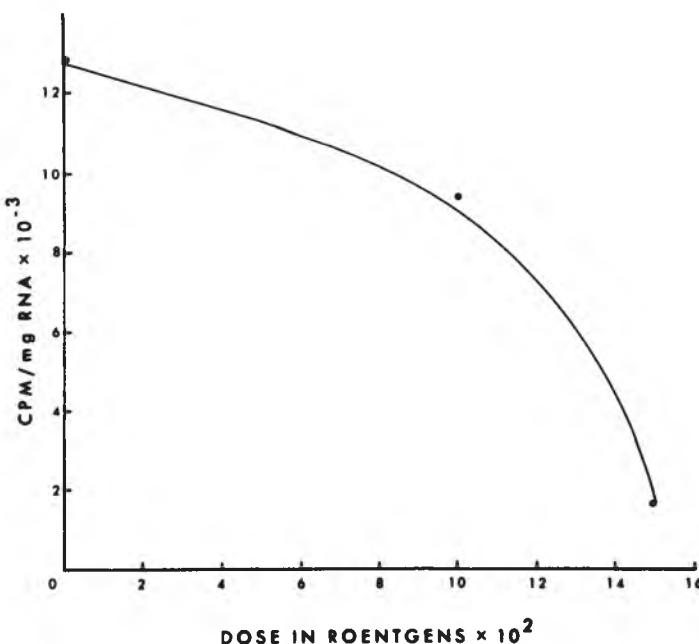


FIGURE 7.2. Effect of X-irradiation on incorporation of ^{14}C -orotic acid in rapidly labeled RNA. The ordinate refers to the counts per minute per milligram of rapidly labeled RNA extracted from the pooled livers of three animals partially hepatectomized 24 hr prior to sacrifice. The abscissa refers to the total-body doses of X-irradiation administered 6 hr prior to sacrifice. (From Uchiyama, T., Fausto, N., and Van Lancertk, J.L., *Arch. Biochem. Biophys.*, 110, 191, 1965. With permission.)

B. DNA/RNA HYBRIDIZATION

Although many investigators have found little or no depression of RNA synthesis in mammalian cells irradiated with less than 2500 rads for periods of 30 min to 6 hr, the question arises of whether RNA synthesized in lethally irradiated cells is different from that in nonirradiated cells. Using the RNA/DNA hybridization technique, it has been reported that the percentage of hybridization, though not showing a significant difference between irradiated and control RNA at doses of 1000 rads and below, shows an increase (30%) at doses of 2500 rads and above. Hybridization competition experiments show that the competition of RNA from nonirradiated cells against labeled RNA from irradiated and nonirradiated cells is similar, implying that there is little qualitative difference in the base sequence of hybridizable RNA synthesized immediately after irradiation.²⁹ The turnover of RNA synthesized in nonirradiated and irradiated cells for 15 min after 2500 or 5985 rads is similar.

IV. EFFECT OF RADIATION ON ENZYMES

Many investigators have studied the effect of irradiation on several enzymes. It has been reported that X-irradiation of mammalian cells increases the activity of some enzymes, whereas it decreases the activity of others. Table 7.1 lists the enzymes that show variable responses after irradiation.

A. DNA ENZYMES

Thymidine kinase and DNA polymerase are important enzymes for the synthesis of DNA. The radiation effects on these enzymes have been investigated in considerable detail. In the

TABLE 7.1
Effect of Ionizing Radiation on the Enzyme Activities of Mammalian Cells

Enzyme	Enzyme Source	Exposure or Dose	Change in Enzyme Activity
Thymidine kinase	Regenerating rat liver	1500 R	Decreases
Thymidine kinase	HeLa cells	2000 rads	No change
DNA polymerase	Regenerating rat liver	1500 R	Decreases
Monoamine oxidase	Mouse and fish liver	2000 rads	Decreases
Tryptophan pyrolase (TP)	Rat liver	Up to 6000 rads	No effect
Hormone-induced pyrolase, TP	Rat liver	Up to 6000 rads	No effect
Substrate-induced pyrolase, TP	Rat liver	Up to 6000 rads	Decreases
L-Serine dehydratase	Rat liver	400–1600 R	Decreases
Dehydrogenase enzymes (succinic, α -glycerophosphate, and lactate dehydrogenase)	Mouse testis	1000 R	Decreases
Alkaline phosphatase, acid phosphatase and β -glucuronidase	Mouse testis	1000 R	Increases
Δ^5 -3 β -hydroxysteroid dehydrogenase	Human placenta	1000 R	Increases
Δ^5 -3 β -hydroxysteroid dehydrogenase	Human placenta	2000 R	Decreases
Tyrosine hydroxylase	Mouse neuroblastoma	600 rads	No effect
Catechol-O-methyltransferase	Mouse neuroblastoma	600 rads	Increases
Acetylcholinesterase	Mouse neuroblastoma	600 rads	Increases
Choline acetyltransferase	Mouse neuroblastoma	600 rads	Increases

rat regenerating liver, the levels of both thymidine kinase and DNA polymerase appear to rise together. The increase in enzyme activity is most pronounced between 18 and 24 hr after hepatectomy, and then declines slowly. In rats receiving 1500 R whole-body exposure 6 hr after hepatectomy, the activities of thymidine kinase and DNA polymerase 24 hr later are reduced to 1 and 20% of controls, respectively. However, when the animals are irradiated 16 hr after hepatectomy, none of the enzyme activity shows any significant change when measured 24 hr after hepatectomy. This indicates that enzymes are more radiosensitive when they are being induced; however, after the completion of synthesis they become highly radioresistant. In contrast to rat regenerating liver, a dose of 2000 rads 1 hr after mitosis did not interfere with the synthesis of thymidine kinase in HeLa cells. The enzyme activity in this cell type rises to a maximum when the cells enter the S phase, but drops rapidly when they enter the G₁ phase. Therefore, the prolonged elevation of the thymidine kinase activity in X-irradiated cells reflects interference with the progression of the cell population to a phase which reduces the enzyme level.¹³ The difference in radiation effect on thymidine kinase of regenerating liver and HeLa cell culture may reflect inherent differences in the control mechanism between the two systems.

B. MONOAMINE OXIDASE (MAO)

Monoamine oxidase is a mitochondrial enzyme and plays an important role in deaminating the biogenic amines. The response of the MAO activity to γ -rays differs from organ to organ within the same species, and from one species to another.⁹ A decrease (71%) in the MAO activity in mouse liver is observed 3 days after 2000 rads; however, in the kidney, heart, and brain, no change in the enzyme activity is seen. The decrease in the enzyme activity parallels the development of radiation sickness. A temporary dose-dependent increase in MAO activity in liver, kidney, heart, brain, and retina of fish is observed immediately after irradiation, whereas in the organs of the mice no such increase is found.

C. LIVER ENZYMES (TRYPTOPHAN PYRROLASE, TYROSINE TRANSAMINASE, AND SERINE DEHYDRATASE)

Liver provides an excellent experimental material for investigating the effects of radiation on the synthesis of specific enzymes. Mammalian liver contains several inducible enzymes, and of these, tryptophan pyrrolase has been studied most extensively. An increase in tryptophan pyrrolase activity can be induced by hydrocortisone and by tryptophan or its analogs. Total-body irradiation blocks the induction by substrate in the normal and adrenalectomized rat, but apparently it has no effect on the induction hormone.⁴⁸ This has been further confirmed in a study in which cortisone-induced rat hepatic tyrosine transaminase and tryptophan pyrrolase were not inhibited by a whole-body exposure of 6000 R. X-irradiation also has no effect on the basal levels of enzymes. Actinomycin D (200 mg/kg of body weight) inhibits hormone-induced enzyme activity by 20%; however, X-irradiation in combination with actinomycin D produces 55% inhibition.⁵⁶ The mechanism of interaction between actinomycin D and X-irradiation in producing greater inhibition of enzyme activity is not known. In another study,³⁰ it has been shown that X-irradiation (800 R) inhibits hormone-induced tryptophan pyrrolase activity in rats, but has no effect on the substrate-induced enzyme activity. It has been suggested that during hormonal induction, less biologically active RNA is synthesized after irradiation. Tryptophan-induced enzyme synthesis does not require the synthesis of new RNA; therefore, no effect of radiation was observed. L-Serine dehydratase,³³ induced by feeding casein hydrolysate, is inhibited by actinomycin D and by total-body exposure (400–1600 R).

A 250-R exposure of ⁶⁰Co to beagle embryos (15 days postgestation) depresses the normal immediate postnatal increase in the activity of glycerol phosphate dehydrogenase and glucose-6-phosphatase in liver, but has no apparent effect on the prenatal level.⁵¹

D. ENZYMES OF THE TESTIS

The main site of dehydrogenase enzymes (succinic, glycerophosphate, and lactate dehydrogenase) is in the interstitial tissue of the testis. After X-irradiation (100 R to the testis), the concentration and distribution of these enzymes change in a very similar pattern. All the enzymes in the interstitial tissue decrease 1 day after irradiation; but the activity of succinic dehydrogenase decreases more than the activity of the other enzymes. The activity returns to the control level 7 days later, but after 56 days the activities of all three enzymes in the interstitial tissue level off at values lower than the controls. Though the testis regenerates morphologically, the activities of the enzymes do not completely return to control values.²⁵ Acid phosphatase activity is found in most cells; after irradiation there is an increase in enzyme activity in interstitial tissue until day 107, and at day 150 it approximates the control value. The alkaline phosphatase activity in interstitial tissue after irradiation also increases.

The β -glucuronidase activity is higher in the tubule than in the interstitial cells. This enzyme activity is nearly absent from the periphery of the tubules, perhaps due to the regeneration of cells at the periphery 15 days after exposure. Surprisingly, at 30 days, the interstitial tissue has considerable enzyme activity, whereas the periphery of tubules has very little enzyme activity. It is interesting to note that the site of β -glucuronidase activity moves from the tubules to the Leydig cells after regeneration. This perhaps confirms the importance of Leydig cell secretions for spermatogenesis.

E. ENZYMES INVOLVED IN THE METABOLISM OF CATECHOLAMINES AND ACETYLCHOLINE

Mouse neuroblastoma cell culture has provided a unique opportunity to study the regulation of neural enzymes. X-irradiation of neuroblastoma cells causes morphological differentiation similar to that produced by dibutyryl cyclic AMP.³⁵ X-irradiation (600–800 rads) also

markedly increases the activity of acetylcholinesterase (AChE),³⁵ catechol-O-methyltransferase,³⁶ and choline acetyltransferase,³⁷ but has no effect on tyrosine hydroxylase.³⁸ The time of increased activity of acetylcholinesterase and choline acetyltransferase corresponds with the time of inhibition of cell division. Many other agents which inhibit cell division without causing morphological differentiation also increase the enzyme activity. Therefore, the activities of these enzymes and morphological differentiation of neuroblastoma cells are independently regulated.

F. EFFECT ON HISTONE ACETYLATION AND METHYLATION

A dose of 1600 rads immediately (<1 hr) produces division delay in the G₂ phase of Chinese hamster ovary cells grown in suspension culture (generation time: 16 hr). Irradiated cells display a postirradiation division delay of 16 hr. Approximately 15% of the irradiated cells complete their first postirradiation division, and only a few complete their second division. DNA and histone synthesis cease between 10 and 12 hr postirradiation. The DNA, protein, and histone contents of the irradiated cell population increase during the division delay period, approximating the levels attained by normal cells in the G₂ phase of the cell cycle. Intracellular levels of histone acetyltransferase and histone methyltransferase rise during the division delay period and fall upon resumption of cell division.⁴³ The methyltransferase system displays a more marked response than the acetyltransferase system.

G. EFFECT ON LYSOSOMAL ENZYMES

Bacq and Alexander⁴ postulated that irradiation releases lytic enzymes within the cells and thereby permits them to attack vital structures. Indeed, specific or total enzymatic activities of most lysosomal enzymes (DNase II, RNase, acid phosphatase, and β -glucuronidase) and lytic enzymes (phosphatase, 5-nucleotidase, and ATPase) increase. These changes are not found immediately after exposure, but develop progressively as a function of time and thus do not represent an immediate effect of irradiation.

Radium treatment of stage II uterine cervical cancer in the human female, at a dose of 3000 rads over a 48-hr period, produces a significant increase in the activity of RNase measured at pH 7.4 in all cytoplasmic fractions of tumor tissue.⁵³ The mechanism for the increase in the enzymatic activity of an irradiated lymphoid organ is a complex one. The following explanations are suggested: (1) change in cell population, (2) change in the rates of synthesis or catabolism of enzymes, and (3) translocation of the enzyme from one organ to another. Change in cell population results from the damage of radiosensitive lymphocytes containing few lysosomes and the persistence of reticulum and macrophages with many lysosomes. These phagocytes start to phagocytose the cellular debris. The average number of lysosomes per cell is thereby increased. The difference between various lysosomal enzymes with respect to the extent and the time course of their activation may be due to the difference in the distribution among various cell types and in the rate of *de novo* synthesis or catabolism of enzymes after irradiation.

V. EFFECT OF RADIATION ON METABOLISM

A. LIPID AND CARBOHYDRATE METABOLISM

Incorporation of various precursors into lipids is enhanced during the first day after an exposure to 300–5000 R. The following factors influence the incorporation of precursors into lipids: (1) dietary state, (2) type of precursor utilized, (3) species, (4) age, (5) radiation dose, and (6) time of assay. Table 7.2 lists some variable factors that affect lipid synthesis after irradiation.

TABLE 7.2
Effect of Some Variable Factors that Affect Lipid Synthesis after Irradiation

Species	Labeled Precursor	Nutritional Status	Exposure (R)	Postirradiation Sacrifice Time	Effect
Rat	Acetate-2- ¹⁴ C	Starved	450	40 hr	Increased
Rat	Acetate-2- ¹⁴ C	Fed	450	6-7 days	Decreased
Rabbit and guinea pig	Acetate-1- ¹⁴ C	Starved and fed	2500	2-3 days	No change

Mouse cells deprived of exogenous fatty acid respond adaptively by increasing the synthesis of fatty acids. X-Irradiation inhibits the induction of fatty acid synthesis. The inhibition in induction (50% for 1000 and 3000 R) indicating that the inhibition of protein synthesis cannot account for the inhibition of induction.⁴⁰ A defect in the fat absorption by the small intestine has also been reported in humans and in animals.

The absorption of carbohydrates by the small intestine is reduced in patients undergoing radiation therapy as well as in animals after whole-body exposure, but the changes do not become pronounced until 2-3 days after irradiation.

The rate of *glycolysis* (anaerobic lactate production) markedly increases in thymocytes after irradiation.⁵⁴ In the brain, the glycolysis rate is not affected, even after 1500 R; however, it does decrease after an exposure of 9000 R.

Glycogen in the rat brain increases³¹ after an exposure of 1500 R; however, the glycogen is enhanced in the livers of animals starved prior to whole-body irradiation (1000 R). The activity of glucokinase in the livers of starved irradiated rats decreases less than nonirradiated starved animals. In rats fed a high carbohydrate diet, the induction of glucokinase is retarded by irradiation. Accordingly, these irradiated animals catabolize glucose to CO₂, or utilize it for glycogen or for lipid synthesis less efficiently than do nonirradiated ones. X-Irradiation has no effect on the utilization of fructose, a carbohydrate phosphorylated by hexokinase but not by glucokinase.²¹

The following interpretations of radiation-induced changes with respect to lipid and carbohydrate metabolism appear reasonable: (1) enhanced lipogenesis and glycogen accumulation occur after irradiation because more substrates of these metabolic processes are made available as a result of tissue breakdown, and (2) substrate oxidation predominates during the postirradiation period because adaptation to the state of starvation in irradiated animals is restricted to nonoxidative enzymes. It should be mentioned that very high exposures of radiation (more than 5000 R *in vivo* and *in vitro*) do not cause an accumulation of glycogen, but rather its degradation. This may result from an increased conversion of phosphorylase *a* to phosphorylase *b*.

B. OXIDATIVE PHOSPHORYLATION

If one employs the P/O ratio (micromoles of inorganic phosphate esterified per microatoms of oxygen consumed) as a criterion of the efficiency of oxidative phosphorylation, one finds a marked depression of this ratio in the radiosensitive organs, particularly those of lymphoid groups after *in vivo* irradiation. Little change in the P/O ratio is noted in relatively radioresistant organs like the liver.⁴² Some investigators have found that P/O in the normal and regenerating rat liver decreases 24-72 hr after total-body irradiation. It is possible that trauma inflicted during isolation and/or storage of mitochondria can reduce the efficiency of oxidative phosphorylation and amplify minor latent damage so that it becomes detectable. Contamination with lysosomal acid phosphatase may considerably lower the P/O ratio by reducing the concentration of the phosphate acceptor.

Mitochondria of certain organs, especially of lymphoid tissue, show a radiation-induced decrease in the P/O ratio only when they have been kept 15–30 min at 0°C, and thus have been exposed to a metabolic stress. Aged mitochondria are obviously no longer normal with respect to their metabolism, particularly because they are apt to leak cytochrome C. Therefore, the data obtained on the P/O ratio after *in vivo* irradiation should be carefully interpreted after taking into consideration various technical and biological factors which might affect the P/O ratio.

In another study, the ability of rat liver mitochondria to oxidize β -hydroxybutyrate, succinate, α -oxoglutarate, pyruvate, and glutamate has been studied after irradiation of mitochondria *in vitro* with exposures of 0 to 100,000 rads.¹⁰ The P/O ratio of mitochondria oxidizing β -hydroxybutyrate and succinate fell by 10% after an exposure of 100,000 rads. After the same dose, the P/O ratio with glutamate or pyruvate fell by 20%, and with α -oxoglutarate by 25%.

Although the oxidative activities and the P/O ratio of the hepatic mitochondria are not altered immediately after irradiation, structural changes (e.g., aggregation and vacuolization) can be detected in rats and mice by means of an electron microscope about 30 min after exposure to several hundred roentgens. Later, in the postirradiation period, swelling of mitochondria and damage to mitochondrial membrane are also noted. The number of mitochondria per cell may decrease after radiation doses of 1000–9600 R.

C. TRACE METAL METABOLISM

Trace metals are either an integral part of enzyme molecules or act as activators or inhibitors of enzymatic reactions. Therefore, trace element determinations are of interest in biological and pathological studies. Changes in the trace metal concentration in irradiated tissues have not been adequately investigated. After a whole-body exposure of 600 R, the concentrations of iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) increase in the rat spleen shortly after irradiation,¹⁹ but return to the control level at 12 days after exposure. The contents of Fe and Mn in the spleen increase after irradiation. In addition, the amount of Cu in the lung increases by about 300% at 8 days, but returns to the control level 12 days after irradiation. The contents of Cu in the lung and Mn in the liver decrease by about 20% 4 days after irradiation. The serum level of Zn in the dog increases (25% of preexposure level) shortly after a whole-body exposure of 200–600 R, and returns to the control level by 108 hr after exposure.³⁹ In the burro, the concentration of plasma iron rises immediately after irradiation, but the concentration of copper does not rise until death, and magnesium concentration decreases. The levels of trace metals in isolated rat mitochondria have been determined after whole-body irradiation (900 rads).¹¹ Increased uptakes of zinc (160% of the control), iron (360% of the control), cobalt (140% of the control), and nickel (160% of the control) are observed 1 day after irradiation, whereas an increase in selenium uptake is not observed until 6 days. Decreased uptakes of manganese (42% of the control) and chromium (79% of the control) are found 1 day after exposure. These data indicate that the transport of certain trace metals across the mitochondrial membrane may be markedly altered by ionizing radiation. The turnover of certain trace metals after whole-body exposure has also been investigated. The turnover rate of ^{54}Mn is faster in the irradiated animals than in the controls, regardless of whether the tracer is administered 48 hr before or 48 hr after irradiation.²³ Therefore, it has been suggested that a process specifically linked to the fundamental events of radiation damage is being mirrored in the metabolism of manganese; however, no further proof has been presented to confirm the above hypothesis.

D. SURFACE CHANGE AFTER IRRADIATION

It has been shown that Burkitt lymphoma cells, derived from the malignant lymphoma of an African child, are very sensitive to irradiation and do not recover from sublethal radiation

damage. It has been assumed that high radiosensitivity and lack of recovery may be related to the fragility of the cell membrane. Indeed, the loss of negative charge after irradiation occurs, and is progressive and irreversible, even after a low exposure of 50 R. This correlates well with the ability of the cell to form a colony.⁴¹

VI. BIOCHEMICAL INDICATORS OF RADIATION INJURY IN MAN

Many biochemical parameters have been measured in human urine after radiation therapy or accidental exposure. Some of these parameters show changes similar to those obtained with animal studies. The deoxycytidine level in human urine markedly increases after radiation therapy treatment.⁷ This finding has been confirmed by several investigators. In rat urine, the increase in deoxycytidine is linear within a whole-body exposure range of 200–650 R; no such increase has been found in human urine after irradiation.

β -Aminoisobutyric acid, a metabolite of thymidine, markedly increases in the urine of irradiated individuals.¹⁵ There is a rise in the level of taurine and β -aminoisobutyric acid (BAIBA) in the urine of accidentally irradiated humans.³ The urine levels of several amino acids, cysteic acid, valine, leucine, hydroxyproline, phenylalanine, arginine, aspartic acid, proline, threonine, and tryptophan markedly increase in irradiated (37–410 R) individuals.^{12,20} In patients who were accidentally exposed at Lockport, the increase in urine levels of amino acids was 20- to 30-fold. In some individuals, no change in the urine amino acid level has been observed following radiation therapy treatment.

Creatine, a metabolite of glycine, is of great importance for the energy metabolism of muscle. An increased urine level of creatine is linear within an exposure range of 50–650 R of X-rays.¹⁶

The release of biogenic amines from various tissues after irradiation has been reported in animals. Although an increase in the urine level of 5-hydroxyindoleacetic acid (5-HIAA) is observed in rat urine after 100 R, no such effect has been seen in 17 female patients who received radiation therapy for genital carcinoma. However, others⁴⁶ have reported that the urinary level of 5-HIAA rises after the local irradiation of patients. The changes in the biochemical parameters described above are not adequate to estimate the extent of the radiation dose received by an individual.

A. IONIZING RADIATION AND GENE EXPRESSION

Ionizing radiation appears to activate diverse nuclear signaling systems. These include protein kinase activation and induction of early response genes. For example, treatment of cells with γ -irradiation is associated with transcriptional activation of the *c-jun* gene.⁵⁸ The *c-jun* gene codes for the major form of the AP-1 leucine zipper transcriptional factor. γ -Irradiation also induces epidermal growth receptor (EGR-1) gene, which codes for a zinc finger DNA-binding protein.⁵⁹ It has been reported that exposure of cells to ionizing radiation is associated with a dose-dependent decrease in histone H₁ gene expressions.⁶⁰ Radiation-induced down regulation of histone H₁ gene expression may be due to its effect at transcriptional and posttranscriptional levels. Irradiation of mammalian cells in culture is associated with increased expression of the TNF (tumor necrosis factor), (PDGF) platelet-derived growth factor, fibroblast growth factor, interleukin-1 (IL-1), and tissue-type plasminogen activator genes.^{61–65} γ -Irradiation of HL-60 human leukemic cells causes rapid activation of protein kinase C (PKC) and also enhances the TNF gene expression, suggesting that radiation-induced enhancement of TNF gene expression is mediated by signal transduction via PKC.⁷⁹ Some studies have demonstrated that the exposure to ionizing radiation is associated with decreases in cyclin A, cyclin B, cdc2, and cdc25 expression, which are considered cell cycle regulatory

genes.⁶⁶⁻⁶⁸ This may, in part, account for radiation-induced growth inhibition. Ionizing radiation induces early expression of the IL-1 β gene.

B. ONCOGENE AND RADIOSENSITIVITY

The availability of plasmid and retroviral vectors carrying specific oncogenes has allowed us to insert exogenous genes into any cell type. The process of gene insertion is often referred to as transfection. The transfection of cells with specific genes can be accomplished by several methods, including electroporation and calcium-phosphate-Co-DNA precipitation techniques. The role of cellular oncogenes or genes in radiosensitivity of mammalian cells can be studied by generating stable transfecants that express high levels of genes under investigation.

Increased expression of *ras* oncogenes has been correlated with increased radioresistance to human and rodent cells in culture.^{70,71} Other oncogenes such as *raf* also play a role in radioresistance.⁷² The combination of high expression of *v-myc* and *H-ras* produces a synergistic effect on radioresistance.⁷³ The membrane localization of *ras*-encoded p21 protein is critical for the maintenance of radioresistant phenotype of mammalian cells in culture.⁷⁴ The microglia assay system is considered a sensitive indicator of chromosomal damage, and a strong correlation between cell death and induction of micronuclei has been repeatedly observed. In a recent study⁷⁵ a dose-dependent association has been noted between radiation-induced formation of micronuclei and *ras* oncogene expression and increased radioresistance to radiation on human osteosarcoma cells in culture.⁷⁵

The presence of the Philadelphia chromosome (Ph1) is characteristic of human myelogenous leukemia (HML). The *abl*/protooncogene on chromosome 9 is fused to *bcr* sequences on chromosome 22, and the juxtaposed sequences transcribe an 8.5-kb hybrid mRNA which codes a 210-kDa *bcr/abl* hybrid protein. HML cell lines expressing *P210 pcr/ab* hybrid gene (32D-PC1 and 32D-LG7) become more sensitive ($D_0 = 1.167$ Gy for 32D-PC1 and 1.27 for 32D-LG7) to γ -irradiation than HML parent lines (32DCl₃ and 32DCl₃ PYN = 1.62 Gy for 32DCl₃; 1.27 Gy for 32DCl₃, PYN).⁷⁶ The increased radiosensitivity may be due to an inhibition of the DNA repair system. On the other hand, the expression of *v-abl*, *c-fms*, or *v-myc* induces radioresistance in the hematopoietic progenitor cell line 32DCl₃ at a low dose rate,⁷⁷ whereas the expression of the *v-src* or *v-abl* oncogene did not alter the radiation response at a high dose rate.⁷⁸ These results suggest that alterations in radiosensitivity induced by certain oncogenes may be dependent upon dose rate.

VII. SUMMARY AND COMMENTS

Ionizing radiation changes the structure, synthesis, and function of nucleic acid and protein. In general, DNA is relatively more radiosensitive than RNA; however, the postirradiation time is very important in comparing the relative radiosensitivity of DNA and RNA. The single-stranded DNA is more radiosensitive than the double-stranded DNA. The initiation of DNA synthesis in the slowly dividing cell is much more sensitive than in the rapidly dividing cell. Some of the damage of DNA can be prevented by prior treatment with SH compounds and dopamine. A supralethal dose of radiation induces DNA synthesis in the G₁ and G₂ phases of the cell cycle, which has been referred to as "unscheduled" DNA synthesis. This has been considered indicative of a repair process. Radiation inhibits the total RNA synthesis, rapidly labeled RNA, and ribosomal RNA. The enzymes are more radiosensitive during the period of induction; they become highly radioresistant after they have completed synthesis. In general, the synthesis of many enzymes (thymidine kinase, DNA polymerase, and monoamine oxidase) is depressed by radiation. The induction of some enzymes by substrate and/or hormone is also inhibited by ionizing radiation. Several enzymes involved in the pathway of catechola-

mine metabolism are elevated in the X-irradiation of neuroblastoma cells in culture. The lysosomal enzymes (DNase, RNase, alkaline phosphatase, and acid phosphatase) also markedly increase after irradiation. The incorporation of various precursors into lipids is enhanced after X-irradiation. The rate of glycolysis also markedly increases in the irradiated thymocytes. The glycogen content increases following X-irradiation. The efficiency of oxidative phosphorylation decreases after X-irradiation. No conclusive study has been made of the effect of radiation on the content and metabolism of trace metals. It has been suggested that the loss of negative charge after irradiation reduces the ability of cells to form colonies. In the irradiated human subject, the urine levels of deoxycytidine, β -aminoisobutyric acid, several amino acids, taurine, and creatine markedly increase. One should remember that all of the above biochemical parameters require strict control, because their levels appear to vary with age, sex, race, and time of assay. Research on the effect of ionizing radiation on the molecular level has increased our knowledge of radiation damage. Some of the recently developed techniques, such as DNA/RNA hybridization and fusion of cells of two different genotypes, may be useful in elucidating the factors that influence the radiosensitivity of cells. X-irradiation is being widely used as a tool in understanding the mechanism of regulation of the enzymes in mammalian cells.

Ionizing radiation activates nuclear signaling systems, which include protein kinases, early response genes (*c-jun*), epidermal growth receptor genes, and H_1 histone genes. Radiation also increases the expression of tumor necrosis factor, platelet-derived growth factor, fibroblast growth factor, interleukin-1, and plasminogen activator genes. On the other hand, ionizing radiation decreases the expression of cell-cycle regulatory genes. When mammalian cells are transfected with *H-ras* and/or *c-myc*, the cells expressing high levels of *H-ras* or *c-myc* show increased resistance to radiation. In hematopoietic progenitor cell lines, the expression of *abl/bcr* hybrid gene is associated with decreased radiosensitivity. The role of *v-abl* and *v-src* in radiosensitivity of cells is independent of dose rate. At a low dose rate, the expression of these oncogenes increases radioresistance of cells, whereas at a high dose rate, it is ineffective.

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Chapter 8

RADIATION SYNDROMES AND THEIR MODIFICATIONS

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I. INTRODUCTION

The signs and symptoms produced by whole-body exposure are referred to as the radiation syndrome. Radiation syndromes have been divided into three major categories: (1) bone marrow syndrome, (2) gastrointestinal (GI) syndrome, and (3) central nervous system (CNS) syndrome. Each syndrome is defined by dose, survival time, and signs and symptoms. There are some differences and some similarities between the radiation syndromes in humans and in animals.

II. BONE MARROW SYNDROME

Some aspects of acute radiation syndromes in man¹ after whole-body irradiation are summarized in Table 8.1. The sources of data on irradiated human beings are as follows: (1) accidents in industry or in laboratories, (2) the Pacific Testing Ground accidents involving exposure to fallout radiation, (3) the experience at Hiroshima and Nagasaki, and (4) medical exposure of patients to whole-body (or near-whole-body) radiation for therapy of cancer or for other reasons. Table 8.2 summarizes the dose estimate of the accidentally exposed individual at various nuclear installations.² It should be pointed out that the radiation factors vary markedly from one accident to another. Thus, a marked variation in the radiation response can be expected. However, the bone marrow damages in the cases were remarkably similar at a comparable dose range. Bond et al.³ have suggested the possible relationship between exposure and survival (Table 8.3).

A. DOSE REQUIREMENT AND SURVIVAL TIME

The dose requirement to produce the bone marrow syndrome varies from one species to another. The bone marrow syndrome is often expressed in terms of ${}^{30}\text{LD}_{50}$ (if a given population is exposed to a ${}^{30}\text{LD}_{50}$ dose, 50% of irradiated individuals will die within 30 days). For most species, the observation time of 30 days is valid because essentially all animals that will die of the bone marrow syndrome die before that time. However, it is necessary to extend the observation period to at least 6 weeks for man and sheep, because an appreciable number of individuals die beyond 30 days. Table 8.4 shows the ${}^{30}\text{LD}_{50}$ values for a number of species.² It appears that the LD_{50} values vary markedly among different species (150–1500 rads). The reasons for this variation in LD_{50} values are unknown.

B. BONE MARROW SYNDROME IN MAN

1. Clinical Signs

The signs and symptoms of acute bone marrow syndrome are caused primarily by radiation damage to the bone marrow. The extensive damage to the lymphatic system and to other radiosensitive organs plays only a minor role in death. Nausea, vomiting, and fatigue are commonly observed after exposure of the whole body. These symptoms are sometimes associated with diarrhea. Infection and bleeding are prominent. The severity of symptoms depends upon the dose. Epilation (loss of hair) occurs in all patients 2 to 6 weeks after exposure. After 6 months, regrowth of the hair is complete. The bone marrow syndrome in humans has been described in great detail by Bond et al.³ and Rubin and Casarett.¹

2. Laboratory Findings

Table 8.5 summarizes the laboratory findings in persons exposed to a bone marrow syndrome dose: initial granulocytosis within the first 2–4 days after exposure, followed by leukopenia during the fourth and fifth weeks. Recovery of leukocytes was observed at day 36. The lymphocyte changes were less variable. Within 3–4 days, the lymphocytes reached their

TABLE 8.1
Some Aspects of the Acute Radiation Syndromes in Man after Whole-Body Irradiation

Aspects	CNS	GI	BM
Chief determining organ	Brain	Small intestine	Bone marrow
Syndrome threshold	2000 R	500 R	100 R
Syndrome latency	1/4 to 3 hr	3-5 days	2-3 weeks
Death threshold	5000 R	1000 R	200 R
Death time	Within 2 days	3 days to 2 weeks	3 weeks to 2 months
Characteristic signs and symptoms	Lethargy, tremors, convulsions ataxia	Malaise, anorexia, nausea, vomiting, diarrhea, GI malfunction, fever, dehydration, electrolyte loss, circulatory collapse	Malaise, fever, dyspnea on exertion, fatigue, leukopenia, thrombopenia, purpura
Major underlying pathology	Vasculitis (CNS), encephalitis meningitis, edema (CNS)	Depletion of intestinal epithelium, neutropenia (marrow damage) infection	Bone marrow atrophy, pancytopenia, infection, hemorrhage, anemia

From Rubin, P. and Casarett, G. W., *Clinical Radiation Pathology*, W.B. Saunders, Philadelphia, 1968, 852. With permission.

TABLE 8.2
Dose Estimate of Accidentally Exposed Individuals at Various Nuclear Installations

Location	Date	Number of Persons Exposed	Dose	Number of Persons Who Died
Los Alamos	1945–1946	10	Several thousand rads to 750 rads (n, γ , soft X-ray)	2 (within 24 hr)
Argonne U.S.S.R.	1952 ?	4 2(2)	159, 126, 61, 11 rads (primarily γ) 450, 300 rads	0 0
Oakridge	1958	8	339, 327, 236, 69, 23 rads (n + γ)	0
Yugoslavia	1958	6	436, 426, 419, 414, 323, 207 rads (n + γ)	1 (at 32 days)
Los Alamos	1958	3	4500 rads (n + γ) 135, 35 rads (γ)	1
Lockport	1960	9	Uncertain	0
Wisconsin	1961	1	250–300 R	0
Hanford	1962	3	Low dose	0
Pacific Testing	1954	267	14–175 R (γ + β)	0

From Prasad, K. N., *Human Radiation Biology*, Harper & Row, New York, 1974, 156. With permission.

minimum and remained at this level for at least 5 weeks. The lymphocyte count remained about 800–900/mm³ during this period. However, the number increased slightly thereafter, but did not return to normal values for weeks and months. The severe decline in platelets was not seen until about 28 days after exposure. After 24 days, the number of platelets dropped below 50,000/mm³. Recovery started at day 32, and the values returned to a normal level after about 7 weeks of exposure. A "late critical phase," which generally occurs during the fourth and fifth weeks, is characterized by a severe granulocytopenia and thrombocytopenia. The "late critical phase" is initiated by nausea, vomiting, fever, and diarrhea. About 50% of the Hiroshima and Nagasaki population exposed to bone marrow syndrome doses died during this "late critical phase." The survival of the exposed individual depends upon recovery of the bone marrow.

C. BONE MARROW SYNDROME IN ANIMALS

The bone marrow syndrome in animals has been well defined. The extent of damage to bone marrow following whole-body exposure varies from one species to another. For example, an exposure of 300 R markedly damages the bone marrow of the dog in a few days, but it produces relatively much less effect on rat bone marrow. At the ${}^{30}\text{LD}_{50}$ level, the bone marrow damage is severe in all species.

TABLE 8.3
Relationship Between Dose and Categories of Radiation Response in Man

Dose (rads)	Categories of Response
500 and above	Survival virtually impossible
200–450	Survival possible
100–200	Survival probable
<100	Survival virtually certain

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 157. With permission.

TABLE 8.4
Radiation LD₅₀ Values for
Different Species

Species	LD ₅₀ (Midline Absorbed Dose, rads)
Sheep	155
Burro	155
Swine	195
Goat	230
Dog	265
Man	270 (?) 243 (?) 225 (?)
Rabbit	840
Mouse	900
Rat	900
Hamster	900
Gerbil	1059
Wild mice	1100-1200
Desert mice	
<i>P. formosus</i>	1300
<i>P. longimembris</i>	1520
Guinea pig	255 (?)
Monkey	398
Marmoset	200

From Bond, V.P., *Comparative Cellular and Species Radiosensitivity*, Bond, V.P. and Sagahara, T., Eds., Igaku Shoin, Tokyo, 1969, 9. With permission.

1. Changes in Bone Marrow Stem Cells

The fact that mammals die due to failure of the bone marrow indicates that a very large proportion of stem cells must be effectively destroyed at this dose level. It has been estimated³ that only 150 rads are needed to reduce the number of stem cells from 100 to 50%. In the mouse, LD₅₀ dose (600 R), or only 2 or 3 stem cells out of 1000, retain their proliferating capacity. Thus, following exposure, only a very few stem cells maintain their ability to proliferate.

TABLE 8.5
Laboratory Findings of Exposed Individuals Whose
Survival Is Considered Possible

Dose (rads)	Granulocytes	Lymphocytes	Platelets
200-500	Initial granulocytosis in most within 2-4 days of exposure; then declines and levels off (2000-3000/mm ³ , recovery starts at day 36)	Less variable changes within 3-4 days reach their minimum (800-900 mm ³) and remain at this level for about 5 weeks; recovery starts thereafter but does not return to normal level for weeks and months	Decrease to minimal level in 8-10 days; level off for a week and then reaches 50,000/mm ³ 24 days after exposure; normal value returned after 7 weeks

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 159. With permission.

2. Changes in Lymphocytes

Although the lymphocyte depression is severe at relatively low doses, it plays a minor role in lethality. In the rat, an exposure of 25 R produces a marked depression in the lymphocyte count. The degree of depression and the rate of recovery are dose-dependent.³

3. Role of Infection

Infection plays a major role in death caused by a bone marrow syndrome dose. High fever and severe infections have been commonly observed in heavily irradiated humans and animals. To estimate the contribution of infection in radiation death produced by the bone marrow syndrome, the radiation responses of conventional and germ-free mice have been compared. In conventional mice (ND-2 strain) a whole-body exposure of 700 R caused 100% mortality with a survival time of 6–8 days, while more than 90% of the germ-free mice survived at this exposure.⁴ Thus, germ-free mice are more radioresistant than conventional mice. The pattern of blood cell changes (granulocytes, platelets, erythrocytes, and lymphocytes) is similar in both groups of animals up to day 12.⁵ The bone marrow and spleen show definite signs of hematopoietic recovery about 8–10 days after irradiation in the germ-free mice, while they do not exhibit recovery in irradiated conventional mice. Death in the conventional mice is associated with thrombocytopenic bleeding and bacterial infection, whereas death in germ-free mice is associated mainly with anemia.

4. Mechanism of Death in Bone Marrow Syndrome

The primary reason for death is bone marrow depletion, with subsequent depletion of blood elements leading to a pancytopenia. Severe neutropenia is associated with the development of infection and platelet deficiency with bleeding. Infection appears to be prominent in all species, but evidence of hemorrhage varies. Hemorrhage is seen in the dog, swine, guinea pig, and man; it is less obvious in the mouse, rat, and rabbit.

5. Effect of High LET Radiation

The RBE values of fast neutrons for bone marrow damage² range from 1 to 2 in the mouse and the rat, but it is 1 in the dog. The time of death in mice markedly varies as a function of LET.⁶ With X- or gamma-radiations, the deaths appear to be confined to the period from about 6 to 20 days — approximately 12 or 13 days. With fast neutrons, an appreciable percentage of deaths occur at the time associated with damage to the GI tract. This phenomenon of early death is more marked after α particle irradiation than after fast neutrons, because the former type of radiation has a greater LET than the latter. This may be due to the fact that high LET radiations in the LD_{50} dose range of bone marrow cause severe GI damage, which contributes to the early death.

6. Effect of Dose Rate

It has been established that the $^{30}LD_{50}$ value increases with a declining dose rate.⁷ In C57BL mice,⁸ the $^{30}LD_{50}$ at a dose rate of 2 R/min was 823 ± 21 R, whereas at a dose rate of 18 R/min it was 624 ± 28 R.

7. Effect of Age

The age at the time of radiation exposure is an important factor to be considered while studying the radiation responses during the bone marrow syndrome in adult animals.^{9,10} The $^{30}LD_{50}$ values for groups exposed at 3 or 7 months of age were 851 and 900 R, respectively. After adjustment of the natural mortality rate, the $^{30}LD_{50}$ values for groups exposed at 17, 21, or 24 months of age were 776, 806, and 747 R, respectively. The mean survival time at the $^{30}LD_{50}$ for the group exposed at 3 months of age was longer than those for groups irradiated

at older ages.¹⁰ These results show that the age of the animal at the time of exposure must be identified when studying the radiosensitivity of animals.

III. GASTROINTESTINAL (GI) SYNDROME

A. GI SYNDROME IN MAN

The signs and symptoms associated with the GI syndrome may be due to the failure of the intestinal mucosa and the bone marrow. Death reflects the synergism of effects resulting from damage of these two tissues. The GI syndrome in man is not as well defined as it is in animals, in terms of the dose-effect relationship. The GI syndrome in the human has been described in great detail by Bond et al.³ and Rubin and Casarett.¹ The initial symptoms resemble airsickness or seasickness and may be influenced by psychological factors and individual susceptibility. The common signs and symptoms include abrupt (within 2 hr) loss of appetite, gastric complaints, and apathy, soon followed by nausea and vomiting, which increase to a maximal intensity at about 8 hr. These symptoms subside as rapidly as they develop; and on the second day, the general health of the exposed individual appears much improved, although nausea and occasional vomiting persist. The symptoms subside on the third day. This clinical condition on the third day may be deceptively encouraging for a day or two before the abrupt onset of the GI syndrome symptoms, including malaise, anorexia, nausea, vomiting, high fever, persistent diarrhea (eventually bloody), and abdominal distention leading to the clinical picture of severe paralytic ileus. During the second week after irradiation, severe dehydration, hemoconcentration, and circulatory collapse are seen, eventually leading to death.³

1. Dose Requirement and Survival Time

The threshold dose of the GI syndrome in man may be about 500 R. A considerable amount of overlapping of the bone marrow and GI syndromes may occur at this dose. Therefore, a higher exposure may be needed to cause death primarily by the GI syndrome. Death usually occurs within 2 weeks of exposure in humans, whereas it occurs within a week in animals.

2. Laboratory Findings

Table 8.6 summarizes the laboratory findings of heavily exposed individuals.³ A marked decrease in granulocyte, lymphocyte, and platelet counts can be seen. The severity of the damage is dose-dependent.

TABLE 8.6
**Laboratory Findings of Exposed Individuals Whose Survival Is
Considered Virtually Impossible**

Dose (rads)	Granulocytes	Lymphocytes	Platelets
4500	Granulocytosis (28,000/mm ³) 14 hr after exposure, only 14,000 at the time of death 32 hr after exposure	Decreased rapidly within hours and disappeared at 6 hr	82,000–92,000/mm ³
1350	Granulocytosis (22,000/mm ³) 3 days after exposure; granulocytopenia (<500 cells/mm ³) at day 6	Disappeared at day 3	Approached zero at day 8
436	11,800/mm ³ at 4 hr and virtually disappeared by day 12	452/mm ³ at 14 hr and thereafter remained low	20% of normal value at day 8

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 159. With permission.

TABLE 8.7
Effect of Abdominal Irradiation on the
Survival of *Necturus Maculosus*

Number of Animals	Exposure (R)	Percent Survivors/30 days
8	0	100
8	4,000	100
8	8,000	100
8	12,000	100
8	20,000	100
8	50,000	100
8	150,000	25

From Prasad, K.N., *Gastrointestinal Radiation Injury*, Sullivan, M.F., Ed., Excerpta Medica, New York, 1968, 381. With permission.

B. GI SYNDROME IN ANIMALS

1. Whole-Body Exposure

The GI syndrome in animals^{11,12} is well defined in terms of the dose–effect relationship. The survival time is relatively short and remarkably constant for most species. Mice characteristically die in about 3.5 days following exposure. Other species, such as the monkey and man, do not die until approximately 7 days after exposure.

2. Partial Body Exposure

Many aspects of the GI syndrome are produced by irradiating the exteriorized segment of the small bowel.¹³ The irradiation of only the small intestine does not cause severe neutropenia. Thus, it would be expected that the infection component of the syndrome would be eliminated. This may be the reason why the GI syndrome produced by irradiation of the small intestine alone requires a higher dose (1800 R) to produce 100% mortality within 2 weeks. The survival time is also longer than that produced by whole-body exposure.¹⁴ *Necturus maculosus* (mud puppy, an amphibian) is highly radioresistant to radiation. An abdominal exposure of 150 kR produced only 25% mortality within 30 days (Table 8.7). After 11 days, the cells lining the villi were decreased in number and exhibited swelling and nuclear fragmentation. Most of the crypt cells appeared normal, but a few showed nuclear fragmentation.¹⁴

3. Role of Infection

Because postirradiation infection is a part of the syndrome, it masks the other pathological effects of ionizing radiation and complicates their study. Consequently, germ-free mice provide an excellent laboratory animal in which radiation syndromes can be studied in the absence of complicating microorganisms. Table 8.8 shows that the survival time in irradiated germ-free mice increases by a factor of about 2. The number of crypt cells in conventional mice was one-third of its normal value in 24 hr, whereas in germ-free mice it does not reach this level until 48 hr. At 3.5 days, only isolated epithelial cells were left in the crypts of conventional mice than for germ-free mice. The intestine of the conventional mouse was denuded until the seventh day after irradiation.¹⁵ The migration time of the cells from the crypt to the tip of the villus was about 48 hr in conventional mice, whereas it was about 96 hr in germ-free mice. This indicates that the rate of cell turnover is greater in conventional mice than that in germ-free mice. This could account, in part, for the difference in the radiation response of the small intestine of conventional and germ-free mice.

TABLE 8.8
Acute GI Syndrome in Conventional and
Germ-Free Mice (CFW)

Type of Animal	Whole-Body Exposure (R)	Survival Time (Range)
Conventional mice	3000	3.5 days (2.9-3.8)
Germ-free mice	3000	7.3 days (6.4-7.7)

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 180. With permission.

4. High LET

The RBE of fast neutrons for the GI syndrome^{3,16} in mice (${}^5\text{LD}_{50}$) is about 1.3-5 — much higher values than those of the bone marrow syndrome. The RBE of fission neutrons for intestinal weight loss¹⁷ on the fourth postirradiation day is about 4.33.

5. Mucosal Changes

The primary changes in the mucosa of mice after 3000 R whole-body irradiation are described in Table 8.9.³ It can be seen that the villi are completely denuded by 3.5 days in mice as well as in the rat, dog, and goat (and probably in the swine); however, denudation does not occur until day 5 or later in the monkey and in man, guinea pig, and hamster.³ There is no evidence of regeneration at death at 3.5 days in mice. Similar changes are also seen in the other segments of the irradiated intestine as well. Cellular depletion, however, does not occur as early as seen in the small bowel, and complete denudation is not observed at the time animals die with the GI syndrome. This may be due to the fact that the small bowel has the most rapid rate of cell renewal, in comparison to other parts of the small intestine. It is also possible that there may be a difference in radiosensitivity between the small bowel and other regions of the GI tract. In the monkey and in man, several focal hemorrhagic lesions in the stomach and colon were found at the time of death by the GI syndrome.³

6. Fluid and Electrolyte Imbalance

Animals with the GI syndrome show severe dehydration. In dehydrated animals, the blood may be so viscous that it is difficult to obtain a blood sample. Severe diarrhea augmented by

TABLE 8.9
Sequences of Histological Changes in the Mouse After 3000 R
Whole-Body X-Irradiation

Area of Intestine	Time after Exposure	Major Changes
Crypt	1st day	Marked decrease in mitotic cells, progressive marked destruction of epithelial lining, associated with pyknosis, nuclear and cytoplasmic swelling and lysis, decrease in cell population, abnormal nuclei
	2nd day	Damage is more pronounced
	3rd day	Majority of crypts essentially devoid of cells
	First 2 days	Villi continue to lose cells and progressively shrink; infiltration of granulocytes into the stroma of villi
Villus	3-3.5 days	Villi completely flattened and a few large epithelial cells may be present; denudation of villi essentially complete at 3.5 days

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 182. With permission.

failure of intestinal absorption and increased leakage into the bowel lumen may be the main factors responsible for the fluid and electrolyte imbalance. However, changes in the fluid and electrolyte balance are generally minimal until the time at which the bowel epithelium becomes denuded and diarrhea commences. In those animals that have a mean survival of 3.5 days, diarrhea begins at approximately 2.5 days. However, diarrhea occurs later in animals that show a longer mean survival time, and begins at about 1–2 days prior to death. In mammals that can vomit (dogs and man), fluid and electrolytes initially may be lost via this route. This is of no significance in the rodent, which is incapable of vomiting. Bile may play a key role in the pathogenesis of diarrhea. Ligation of the bile duct of the irradiated rat or cannulation and diversion of bile prevents diarrhea.¹⁸ Reinstillation of liquid bile or bile salt may cause diarrhea to reappear. Electrolytes are lost primarily as a result of diarrhea. The loss of sodium over the first 4 days is much more marked than that of potassium.¹⁹ The sodium loss is largely via the small intestine.

7. Infection

Infection does play a role in the GI syndrome and the resulting mortality. A marked neutropenia by days 2–3 increases the susceptibility of the animals to infection. The role of bacteria in the GI syndrome is further shown by the fact that the irradiated germ-free mice live longer than irradiated conventional mice. Fever is prominent in both dog and man prior to death with the GI syndrome. Fever is not present in the irradiated mouse or rat; however, the normal mouse does not develop fever when infected.

8. Nutritional Impairment

The loss of weight during the GI syndrome is marked. Reduced intake may be the major cause of the weight loss. Starvation, in itself, is not a major factor in death caused by the GI syndrome. Intestinal absorption decreases progressively with time during the GI syndrome, reaching a minimum at day 3. The decrease in absorption is primarily due to the loss of epithelial cells from the villus.

9. Changes in the Vascular System

There may be an apparent relationship between the degree and rate of development of radiation injury of the mucosal epithelial cells and the degree and time of development of lumen blockage of the fine vasculature.²⁰ Thus, the damage to blood vessels may be important in the pathogenesis of the GI syndrome produced by a high exposure (1460 R).

10. Mechanism of Death

The denudation of villi is considered a major factor in the cause of death; however, other factors such as infection, hemorrhage, fluid imbalance, and electrolyte imbalance also contribute to serious illness and death.

IV. CENTRAL NERVOUS SYSTEM (CNS) SYNDROME

A. CNS SYNDROME IN ANIMALS

1. Signs and Symptoms

The CNS syndrome in man is poorly defined, therefore animal data are primarily discussed. A whole-body exposure of about 5000 R is considered a threshold dose in man. The survival time is dose-dependent. The larger the dose, the shorter the survival time. The symptoms produced are primarily due to CNS damage. CNS syndrome in man is very well described by

Rubin and Casarett.¹ The CNS syndrome is characterized by periods of agitation and apparent marked apathy, followed by signs of disorientation, disturbed equilibrium, ataxia, diarrhea, vomiting, opisthotonus, convulsion, prostration, coma, and death. The time of onset and progression of subsequent signs and symptoms are dose-dependent.

2. Histopathological Changes

Perivascular and parenchymal granulocytic infiltration in the meninges, choroid plexuses, and brain are often seen after head or whole-body exposure of about 5000 R or more.²¹ The extent of cellular infiltration is dose- and time-dependent. The early infiltrates contain granulocytes, whereas after 12–14 hr they contain mostly mononuclear cells and, later, debris-filled macrophages.

a. Vasculitis

Vasculitis is a common observation in the monkey surviving 3 hr or more following 5000 R of whole-body or head irradiation. The early lesion consists of focal surrounding tissue. Veins and arteries of all sizes are equally involved. Vasculitis is a prominent alteration throughout the cerebrum, particularly in the paraventricular area. The cerebellum, brain stem, and spinal cord are qualitatively less involved. The lesions appear earlier in the gray matter, but eventually the white matter becomes more severely involved.³ The extent of the infiltration is biphasic, with peaks at 8 and 48 hr after exposure. At a high exposure of 30,000 R to the head only, the 8-hr peak involvement is absent. At 10–13 hr, the granulocytic infiltrations are associated with edema and small lesions in the adjacent white matter.³

b. Meningitis

Meningitis is seen in about two-thirds of the monkeys receiving between 5000 and 30,000 R of whole-body exposure. It appears as early as 2 hr after head exposure of 10,000 R. Later, the perivascular granulocytic infiltrates in the meninges and foci of exudates over the sulci increase, reaching a maximum during the next 24 hr. After this peak, the infiltrates decrease somewhat, but are found as long as 96 hr after irradiation.²²

c. Choroid Plexitis

The choroid plexuses are also involved, with a maximum peak of edema and leukocytic infiltration occurring 8 hr after exposure. This subsides considerably, but the infiltrates persist at 96 hr after irradiation.²² The local brain damage probably results from the damage of cells as well as the injury of blood capillaries.

3. Cytological Changes

a. Cerebellum

In many species pyknosis of neuronal cells of the cerebellum occurs in a dose-dependent fashion. However, in the monkey, even after 30,000 R, approximately 10% of the irradiated animals may not show pyknosis of neurons. In the rabbit, the threshold dose for pyknosis of neurons is between 4000 and 5000 R.²³

b. Cerebrum

Neurons of the cortex show few, if any, morphological changes during the CNS syndrome.¹ Pyknotic and lytic changes in neurons as well as neuronophagia and satellitosis of the brain stem medulla and hypothalamus have been observed after head irradiation in the monkey.²⁴ The brains of these animals were severely edematous. The cellular changes seen can result from postmortem changes alone, and they can be seen in the brain rendered edematous from causes

other than irradiation, when examined at a similar time following death.²² Thus, it is difficult to assess the specificity of radiation-induced damage in the brain during the CNS syndrome.

4. Vascular Changes

Damages to blood vessels and blood capillaries are commonly seen in the brain during the CNS syndrome.¹ These changes include hemorrhage, capillary endothelial vacuolization, vasculitis, and capillary permeability. Vascular changes probably contribute to local cellular damage; however, whether vascular damage is the primary cause of death produced by the CNS syndrome is unsettled.

5. Cause of Death

Death probably results from neuronal change, either directly or via increases in intracranial pressure.¹

V. MODIFICATION OF RADIATION SYNDROMES

The radiation response in the whole organism can be modified either by preirradiation or postirradiation treatment. The various agents that modify the radiation response are discussed under the following categories: (1) radioprotectors and (2) radiotherapeutic agents or procedures.

A. RADIOPROTECTORS

Radioprotectors are substances or procedures that, when administered before X-irradiation, protect animals against radiation damage. Radioprotectors do not reduce radiation damage when given after irradiation.

1. Partial Body Shielding

Partial shielding of any part of the body at the dose range of the LD₅₀ significantly reduces radiation damage. The shielding of the area rich in bone marrow is relatively more effective, because the undamaged bone marrow supplies new cells to the damaged portion of the body, and therefore, bone marrow depression may not be severe enough to precipitate death.²⁵ The LD₅₀ value for the unshielded rat is 600 R, for the shielded abdomen and thorax it is 900 R, and for only the head shielded it is 700 R. Shielding the exteriorized spleen with lead during whole-body X-irradiation raises the ³⁰LD₅₀ value in mice from 550 to 1100 R.²⁶ The efficacy of spleen shielding varies from one species to another, being best in young mice.

2. Effect of Oxygen

The oxygen effect in animals should be interpreted with caution. The oxygen tension between cells appears to vary. Oxygen tension between the cells in the bone marrow is very low (10 mm Hg or less), but comparatively high in the subcutaneous tissue.²⁷ In addition, when the animal is deprived of oxygen a number of reflex reactions take place;²⁸ in particular, there is an intense stimulation of the sympathetic nervous system, which liberates considerable quantities of adrenaline and noradrenaline from the adrenal medulla, and also of the endings of the adrenergic nerves. These two amines are good radioprotectors.²⁸

If rats are subjected to an atmosphere containing only 5% oxygen instead of 20% (the concentration of normal air), their sensitivity to radiation decreases by about half; however, in mice the radioprotective effect of hypoxia is less than that in rats.²⁹ It has been suggested that variations in oxygen tension may contribute to the high radiosensitivity of young mice.³⁰ The higher degree of protection²⁹ against radiation damage in animals breathing 5% oxygen

TABLE 8.10
Effect of Hypoxia on Survival
of X-Irradiated Rats

Radiation Exposure (R)	Percent Survival	
	Air	5% O ₂
600	63	100
800	0	100
1000	0	91
1200	0	81
1400	0	29

Note: The data were summarized from Dowdy, A.H., Bennet, L.R., and Chastain, S.M., *Radiology*, 55, 879, 1950.

(Table 8.10) is interesting, because mammalian cells in culture do not show any significant change in radiation response under the above conditions.²⁸ Probably a portion of cells in the bone marrow or in the intestine is normally in equilibrium with a relatively low concentration of oxygen, so that a reduction of oxygen in the atmosphere makes these cells completely anoxic.

3. Effect of Temperature

The radiosensitivity of rats³¹ markedly decreases when they are irradiated under deeply chilled conditions. The ${}^{30}\text{LD}_{50}$ value is raised from 650 to 1760 R when mice are irradiated while their colonic temperature is between 0 and 0.5°C.³² At these temperatures, mice could survive only 30 min. Anoxia may be the most important factor in the mechanism of protection afforded by low temperature, because when infant mice are irradiated at a body temperature of 37°C, the addition of nitrogen does not increase protection; whereas the addition of oxygen reduces survival considerably.

Certain mammals, such as ground squirrels and doormice, undergo hibernation. When these animals are irradiated in the hibernating state, the expression of radiation damage is considerably delayed; but as soon as the animals are allowed to wake up, the symptoms of radiation injury develop just as in the nonhibernating controls. Thus, survival time in hibernating animals after irradiation is increased, but the absolute survival is unaffected.

4. Effect of Radioprotective Chemicals

The basic purposes of the search for radioprotective chemicals have been twofold: (1) to protect the normal tissues preferentially during radiation therapy of malignant tumors, and (2) to obtain a greater knowledge of the mechanism of radiation damage. In spite of extensive work, the first objective remains elusive. The dose of a radioprotective chemical required for protection is highly toxic in humans. These agents protect normal and malignant cells to about the same extent. However, studies involving radioprotective agents have increased our knowledge of the mechanisms of radiation damage. There are hundreds of compounds having varying degrees of radioprotective value. These have been extensively described in a separate textbook.³³ Here, this subject is dealt with in brief.

5. Factors to Be Considered in the Study of Radioprotective Agents

When testing the radioprotective efficacy of a new compound, the following factors must be considered: (1) toxicity of the agent, (2) choice of radiation dose, (3) choice of experimental animal, (4) route of drug administration, and (5) preexposure time.

a. Toxicity of the Protective Agent

It is essential that some idea of the toxicity of the compound be established before it is tested as a radioprotective agent. A nontoxic dose of a drug should be used in the radioprotective study.

b. Radiation Dose

The total whole-body exposure should be in the range of the $^{30}\text{LD}_{50}$ dose. In addition, if the efficacy of the drug is small, this can be masked when a high exposure is used.

c. Experimental Animal

Generally, rodents are very useful in evaluating the radioprotective efficiency of the compound; the same compound may be ineffective in large animals, because the concentration that is expected to provide protection is lethal in those animals. For example, in mice, cysteamine (100 mg/kg of body weight) provides considerable protection against radiation damage, but the same concentration of drug is lethal in the dog. The concentration of cysteamine that is safe for the dog is too low to be of any radioprotective value. Therefore, rodents may be an ideal choice for an initial study of radioprotective agents. The animals must be free from infection, because the mortality of irradiated animals in the presence of infection is greater than in the noninfected irradiated controls. Therefore, the radioprotective effect of an agent may be masked in the infected animals.

d. Route of Drug Injection

The route of injection markedly influences the protective action of a compound, because the rates of absorption, distribution, and excretion of the compound vary depending upon the route of administration. Generally, the drugs are injected intraperitoneally (i.p.) because this technique is easiest to use with small animals. However, the rates of absorption, distribution, and excretion are most rapid when drugs are injected intravenously (i.v.), and least rapid when injected orally. The route of administration also affects the toxicity of the compound; 2-mercaptoproethylamine (cysteamine) and its oxidation product, bis-(2-mercaptoproethyl) disulfide (cystamine), are equally effective after an i.p. injection. However, cystamine is also effective when given orally, whereas cysteamine is not. The protective concentration of cystamine, when injected i.v., produces a fall in blood pressure that is generally fatal to mice.

e. Pre-exposure Time

The pre-exposure time, which provides maximal protection against radiation damage, varies markedly from one compound to another. Some compounds are effective when given immediately before whole-body exposure, whereas others are ineffective under the above experimental condition. Therefore, one must determine an optimal preexposure time for each radioprotective compound.

6. Criteria of Evaluation

The following criteria may be used to evaluate the radioprotective effect of a compound:

1. Dose reduction factor (DRF), using the $^{30}\text{LD}_{50}$ primarily as an end point

$$\text{DRF} = \frac{\text{Dose to produce an effect in the presence of a compound}}{\text{Dose to produce the same effect in the absence of a compound}}$$

2. Percent survivors as a function of radiation dose when the drug dose (often a maximal) is constant

3. The efficacy of the radioprotective agents against a high dose rate and a high LET
4. The efficacy of the radioprotective agent against a late effect of radiation such as life shortening, cataract formation, chromosomal aberration, and carcinogenesis

Radioprotective agents have been divided²⁷ into four major groups: (1) thiols, (2) other sulfur compounds, (3) pharmacological agents, and (4) other protective agents.

7. Thiols

The thiols include cysteine, 2-mercaptopropylamine (cysteamine), cystamine, aminoethylisothiourea dihydrobromide (AET), and 2-mercaptopropylguanidine (MEG). Sulfhydryl (SH) amines are strong reducing agents at physiological temperature and pH. The DRF values for various compounds varies from 1.4 to 2.0. Other features of these compounds are described below.

a. Cysteine

The DRF value of cysteine on the criteria of the ³⁰LD₅₀ for rats (1.5) is less than that for mice (1.7). The DRF values on other criteria, such as loss of spleen weight, lymphopenia, and granulocytopenia, are roughly the same. Both the L-isomer and D-isomer of cysteine are equally effective.³⁴

b. Cysteamine

Cysteamine (150 mg/kg) produces the same degree of protection as 1200 mg/kg of cysteine. In humans, cysteamine (3–4 mg/kg), when given i.v., failed to prevent postirradiation leukopenia or radiation sickness.³⁵ Cysteamine provides some protection to the mouse and rat fetus exposed *in utero* by irradiation of the mother. This compound also protects against radiation-induced sterility in female mice, but not against damage to the seminiferous epithelium. This is probably because very little of the drug reaches the testis.³⁶

c. Aminoethylisothiourea Dihydrobromide (AET)

The DRF values of AET given i.p. vary from 1.45 to 2.10. The DRF value of AET given orally is no better than 1.2. The DRF value for the damage of lymphatic tissue and bone marrow is about 2.0. AET and its derivatives are effective in mice, rats, and monkeys,³⁷ but are of little value in dogs.³⁸ Its ineffectiveness in dogs may be attributable, in part, to the high toxicity of this compound. AET is highly toxic in the human. Nausea and vomiting are commonly found after either oral or i.v. doses of only 10–20 mg/kg, and in some patients, circulatory disturbances are also observed.³⁹ AET also inhibits the carcinogenic and life-shortening effects of radiation in animals.⁴⁰

d. 2-Mercaptopropylguanidine (MEG)

The neutralization of AET to pH 7 quantitatively converts the compound to MEG. The oxidation product of MEG is bis-(2-guanidinoethyl) disulfide (GED). The DRF value of MEG at 275 mg/kg is about 1.7, and GED at 200 mg/kg is about 1.4.⁴¹

8. Mechanism of Protection by SH Compounds

The following mechanisms of radioprotection by SH compounds have been suggested.

a. Free-Radical Scavenger

The SH compounds inactivate free radicals formed by irradiation; however, several compounds such as sodium citrate and norepinephrine, which inactivate free radicals *in vitro*, have no radioprotective value *in vivo*.

b. Production of Hypoxia

The SH compounds produce tissue hypoxia, which may be one of the important factors in radiation protection.

c. Formation of Mixed Disulfide Bonds

The SH compounds form a mixed disulfide bond with the tissue protein containing the SH group.⁴² These disulfides are transient and are reduced by glutathione reductase. The SH groups of the radioprotective compound chemically shield the SH groups of the tissue proteins, which are essential for the cellular functions. This hypothesis presumes that cellular proteins are the most radiosensitive targets for cell survival. However, the DNA molecule in the cell is considered the most radiosensitive target. Therefore, the proposed hypothesis may not be valid.

d. Reversible DNA Inhibition

The SH compounds reversibly inhibit DNA synthesis, which may delay DNA replication. This, then, allows time for the DNA molecule to repair its damage.²⁸ If damaged DNA molecules are allowed to replicate, the broken strands will fall apart and no repair will occur.

9. Combined Effect of Thiols and Hypoxia

Hypoxia (8% oxygen) and three SH compounds (cysteamine, cysteine, and cystamine), by themselves, increased the survival of irradiated mice (1100 R) from 0 to about 20%.³⁴ The combination of hypoxia plus any of the thiols increased survival to about 65%. Therefore, these agents may, in part, have different mechanisms of protection in mice.

10. Other Sulfur-Containing Compounds

Several hundred compounds containing sulfur have been tested, but only a few show any significant protective action. Some of the sulfur-containing compounds include thiourea, thiouracil, dithiocarbamates, dithiooxamides, thiazolines, sulfoxides, and sulfones. Dimethyl sulfoxide has a DRF value of 1.33 for the same strain of mice in which the DRF value of AET is about 1.45.

11. Pharmacological Agents**a. Anesthetic Drugs and Alcohol**

Drugs commonly used as anesthetics are not effective in radiation protection. Ethyl alcohol, when given in large amounts (6–7.5 ml/kg i.p. in a 10 or 25% aqueous solution), provides protection against X-irradiation in mice. This concentration (7.5 ml/kg), if translated from mouse to man, would correspond to about a quart of 100-proof whiskey, an amount frequently considered lethal if ingested all at once. Respiratory depression after alcohol ingestion is marked and leads to tissue hypoxia, which may be one of the most important mechanisms of radiation protection.

b. Analgesics

Morphine (60 mg/kg) and heroin (60 mg/kg) increase the ${}^{30}\text{LD}_{50}$ in mice from 609 to 830 R.³³ Sodium salicylate (600 mg/kg) increases the survival at 700 R from 0 to 50%.

c. Tranquilizers

Chloropromazine is ineffective. Injection of reserpine (4 mg/kg) 12 hr before exposure raises the ${}^{30}\text{LD}_{50}$ for male mice from 605 to 825 R, and for females from 635 to 727 R. Reserpine is ineffective in rats.

12. Cholinergic Drugs

Acetylcholine, metacholine (acetyl- β -methylcholine), and carbaminoylcholine are of some radioprotective value in mice, but choline is ineffective.³³

13. Epinephrine and Norepinephrine

Epinephrine (5 mg/kg) protects animals against radiation death, whereas norepinephrine does not.²⁸ This is due to the fact that tissue anoxia is not produced by a comparable dose of norepinephrine. For example, equal doses (1.5 mg/kg) of both drugs raised the arterial blood pressure to approximately the same extent, but norepinephrine decreased the oxygen tension of the spleen by only 48%; whereas epinephrine decreased it by about 90%. The former permitted the survival of only 5% of the irradiated animals; the latter produced 95% survival.³³ Tyramine is less effective than epinephrine. Ephedrine (80 mg/kg) is ineffective. Methoxamine (80 mg/kg) increased the ${}^{30}\text{LD}_{50}$ in rats from 825 to 1100 R, a DRF value of about 1.3.⁴³

14. Dopamine

Dopamine (400 mg/kg), when given immediately before whole-body exposure, protected 80% of irradiated mice at an exposure (700 R) that produced 100% lethality.⁴⁴ The radioprotective efficacy of dopamine did not decrease in splenectomized mice. L-Dihydroxyphenylalanine (dopa), a precursor of dopamine, was ineffective. Dopamine⁴⁵ also protected rats against radiation damage. The DRF value for dopamine was about 1.3. The exact mechanism of dopamine protection is unknown; however, the following possibilities have been suggested:

1. Dopamine protects DNA *in vitro* against radiation damage,⁴⁶ probably via the free-radical scavenger mechanism. Because the concentration of this catecholamine in the spleen and small intestine is high, the free-radical mechanism *in vivo* may be a factor in the mechanism of protection.
2. Dopamine causes reversible inhibition in the DNA synthesis of mouse spleen.⁴⁷ It is presumed that the inhibition of DNA synthesis may delay DNA replication and thus enhances the probability of the repair of radiation damage.
3. Unlike epinephrine, dopamine protects splenectomized mice.⁴⁴
4. The effectiveness of dopamine in protecting irradiated animals exists for a very short time; therefore, it is unlikely that the radioprotective action of dopamine is exercised via epinephrine.

Hypoxia does not appear to be a major factor in dopamine protection.

15. Histamine

The DRF value for histamine (500 mg/kg) in CBA mice was about 1.5 while in the C57BL strain it was only 1.1.³³ The oxygen tension in the spleen after histamine treatment decreased by 77–93%. Therefore, the decreased oxygen tension may be the major mechanism of protection.

16. Serotonin

Serotonin (25 mg/kg i.v. or 95 mg/kg i.p.) is as effective as cysteamine in mice. The DRF value of serotonin (50 mg/kg) is about 1.84.²⁷ The combination of serotonin with AET is more effective than either agent alone.³³ The radioprotective efficacy of serotonin markedly decreases in mice irradiated with fast neutrons.⁴⁸

17. Hormones

Very little work has been done involving the administration of hormones a few minutes before exposure. Slight sex differences are consistently noted in most species, females being slightly more resistant than males. After castration the situation is reversed. The resistance of castrated male mice to radiation can be increased by the administration of testosterone, but estradiol produces no such effect in spayed females.³³ Adrenalectomized rats are relatively more radiosensitive. The DRF value of hormones, such as the adrenal hormones and thyroid, is about 1.1.³³ Estrogen and colchicine are also of some radioprotective value.³³ Both of these agents produce transient leukopenia, which in the recovery phase results in leukocytosis. When the animals are irradiated during this period of leukocytosis, the survival of irradiated animals is increased.³³ The melanocyte-stimulating hormone (1 mg/mouse), when given before X-irradiation, is of some radioprotective value.⁴⁹

18. Other Radioprotective Agents

a. Cyanide

Sodium cyanide (5 mg/kg) is of some radioprotective value.²⁸

b. Nucleic Acid Derivatives

Nucleic acid derivatives such as uracil, 5-hydroxy-4-methyluracil, 5-amino-4-methyluracil, and 5-amino-4-methylcytosine are of some radioprotective value.³³ They increase the survival at the ${}^{30}\text{LD}_{50}$ value by 20–40%. Adenosine triphosphate (ATP) also raised the ${}^{30}\text{LD}_{50}$ value by about 30%. When ATP and pyridoxal-5-phosphate are administered together, the ${}^{30}\text{LD}_{50}$ value is increased by about 60%. Adenosine monophosphate by itself showed a DRF value of 1.55, and with pyridoxal-5-phosphate a DRF of 1.7.

c. Sodium Fluoroacetate

Sodium fluoroacetate (7.5 mg/kg), when injected i.p. in mice 3 hr before X-irradiation, increased the average ${}^{30}\text{LD}_{50}$ value from 648 to 998 R.³³

d. Para-Aminopropiophenone (PAPP)

PAPP (40 mg/kg), injected i.p. 15 min before X-irradiation, produced a DRF value of 1.7 for the bone marrow syndrome, 1.4 for the GI syndrome, and 2.28 for the CNS syndrome.⁵⁰ The mechanism of protection is primarily via tissue hypoxia.

e. Melittin

The chief component of free venom is melittin, a strongly basic polypeptide of molecular weight 2850. A dose of 5 mg/kg, injected s.c. 24 hr before X-irradiation, produced 100% survival at a ${}^{30}\text{LD}_{50}$ value.⁵¹ The mechanism of protection is unknown.

f. Endotoxin

Radiation depresses hematopoiesis and the immune system, whereas endotoxins stimulate bone marrow. When the typhoid-paratyphoid vaccine (TAB) containing 1.5×10^9 killed organisms was injected in mice 24 hr before X-irradiation, a maximum protection was observed.⁵² The time of injection is very important for the radioprotective effect of bacterial endotoxins.

g. Imidazole

Imidazole (350 mg/kg) increased the survival of irradiated mice from 14 to about 80% when the drug was given 5 min before irradiation.⁵³

h. Adenosine 3',5'-Cyclic-monophosphate (Cyclic AMP)

Cyclic AMP-stimulating agents, when given before X-irradiation, protect mammalian cells in culture.^{54,55} They also protect normal hair follicles⁵⁶ and the gut stem cells^{56,57} of mice against radiation damage. However, they do not protect tumor cells *in vivo*.⁵⁶ However, in subsequent studies, cyclic AMP did not produce differential protection in other tumors.

19. Radioprotective Agents and High LET Radiation

The effectiveness of radioprotective agents is markedly reduced when used with radiation of a high LET. This may be due to the fact that the radioprotective agents mostly protect animals against the indirect effect of radiation. However, high LET radiation causes damage mostly by a direct effect (ionization).

B. RADIOTHERAPEUTIC AGENTS AND PROCEDURES

The radiotherapeutic agents may be grouped into two broad categories: (1) chemical agents and (2) biological agents.

1. Chemical Agents***a. Antibiotics***

Because one of the features of mice dying after irradiation is severe infection, the injection of antibiotics (penicillin and streptomycin) whenever infection is indicated prolongs the survival time of irradiated animals, and may even save the animal if the whole-body exposure is low. The effectiveness of the antibiotics is observed only in the dose range of the bone marrow syndrome.

b. Imidazole

Imidazole (350 mg/kg), when given immediately after exposure, raises the survival from 14 to 42%.⁵⁸

c. Lipid

Olive oil, injected i.p. 30 min after irradiation, increases the survival of irradiated mice (³⁰LD₅₀) from 50 to 87.5%.³³ Diets containing either cottonseed oil or pure methyl linoleate were beneficial to chronically irradiated rats.³³ Lycopene, the carotenoid constituting the principal pigment in tomatoes (1 mg/kg), injected 30 min after whole-body exposure (725 R), increased the survival of mice from 20 to 68%.³³ Larger doses or a longer time interval had no therapeutic value.

d. Nucleic Acid Derivatives

The administration of DNA and RNA had a therapeutic effect on irradiated mice.³³ Both heterologous and homologous RNA had the same efficiency,³³ and high-molecular-weight nucleic acids exerted a greater therapeutic effect than the nucleic acids of lower molecular weight.^{33,59} The treatments (3–6 hr after irradiation) raised the survival of irradiated mice from 50 to 85%.

e. Hormones

The administration of the adrenocorticotropic hormone (ACTH) increased the survival of irradiated animals (600 R) from 17 to 56% in 7- to 9-day-old rats. In older animals, the adrenals are presumably maximally stimulated, and therefore, additional ACTH has no therapeutic effect. Parathyroid extract, free of protein, provides a considerable degree of protection against radiation damage when given after X-irradiation.⁶⁰

f. Erythropoietin

The survival of irradiated mice (1000 R, whole-body) increased from 0 to 80% when erythropoietin was injected after irradiation.²⁵ The erythropoietin dose (10 units) injected 1 hr after irradiation (651 R) produced a DRF value of 1.12.⁵⁸

2. Biological Agents**a. Parabiosis**

An irradiated parabiotic pair is defined as a pair in which one partner is irradiated while the other is shielded with lead during exposure. In this procedure, two animals, usually rats, are joined side by side by sewing together skin incisions. As a result, cross-circulation between the two animals is established through capillaries of the skin. The nonirradiated partner provides the irradiated partner with enough blood elements so that no hematopoietic failure occurs, and the animals survive the lethal dose.⁶¹

b. Spleen and Spleen Extract

Implantation of the spleen into the peritoneal cavities of the irradiated mice enhances survival.⁶² If the implantation is carried out within 2 hr after exposure, the survival after whole-body exposure of 1025 R increases to about 50%. When spleen implantation is performed 1–2 days after exposure, the number of survivors is only 24%. The requirements for the therapeutic effect from the implanted spleen are the following: (1) four syngeneic spleens from young mice (7–12 days old) are effective — two spleens are ineffective, (2) age of the donor is also important — young mice spleens give better results — implantations of spleens from 12-week-old mice have no therapeutic value, and (3) vascularization of the spleen is also very important. The injection of spleen cells after whole-body exposure in mice provides considerable protection; however, the protective effect of spleen cells decreases with the age of the spleen donor. The i.v. route is more effective than the i.p. one. When syngeneic (cells of donors genetically identical to recipient) spleen cells (5×10^6 cells) were injected i.v. into irradiated mice, 100% survival was obtained at a dose that produces 100% lethality.⁶³ The number of allogeneic spleen cells needed to provide significant protection was much greater than that of syngeneic cells. Allogeneic (cells of donors genetically different from recipients — two animals may belong to the same species but have a different genetic makeup) spleen cells (50×10^6 cells), when injected i.v., provided only 55% protection for a 30-day period; however, all survivors died soon afterward. This second mortality was related to the immunological problem created by allogeneic cells. A cell-free extract of spleen, when injected i.p. after a whole-body exposure, increases the survival of irradiated mice by 10–40%. The administration of syngeneic, allogeneic, and xenogeneic (cells of donor genetically different from recipient — two animals may belong to different species or genus) spleen extract provides a similar degree of protection.⁶⁴ A cell-free spleen extract is effective when given i.v., i.p., or i.m. A higher degree of survival of irradiated animals can be obtained when the spleen extract is purified. The second protein peak (molecular weight = 70,000),⁶⁵ obtained by gel filtration with Sephadex G-200, increased the survival of irradiated mice by 94%.

c. Bone Marrow Transplantation

The i.v. injection of syngeneic bone marrow (5×10^6 cells) immediately after exposure prevented death in 75% of mice exposed to 900 R. Intravenous administration provides the best protection.³ Allogeneic and xenogeneic cells require at least ten times more cells to provide a significant protection; however, these animals later die of secondary disease. Allogeneic cells are more effective at the ${}^{30}\text{LD}_{100}$ than at the ${}^{30}\text{LD}_{50}$, because a higher dose suppresses the host immunity and thereby allows the acceptance of a graft; i.p. injection

requires 70 times more cells than i.v. for a similar degree of protection. Under optimal conditions, the injection of syngeneic bone marrow will double the ${}^{30}\text{LD}_{50}$ value. There is no recovery of the mouse embryo irradiated *in utero* when bone marrow is administered to the mother after exposure to 200 R.³³ This may be due to the fact that the hematopoietic system is not developed in these embryos (8.5 days after fertilization) and hence could not be involved in radiation death. If one is using allogeneic or xenogeneic cells, a 24-hr postirradiation time interval is favored for the following theoretical reasons: (1) most of the cell debris are removed by this time, and (2) the immunological response to soluble antigens is minimum at this time.

d. Transfusion of Peripheral Blood

Transfusion of leukocytes improved the survival of dogs exposed to the ${}^{30}\text{LD}_{50}$.³ When 5–11 transfusions of leukocytes were given, 85% of the irradiated dogs survived. Administration of platelets was even more effective than the leukocytes, because only 3–5 transfusions were needed to provide a similar degree of protection. Fresh platelets were more important in controlling hemorrhage than lyophilized ones.

e. Injection of Nonhematopoietic Cells

A suspension of whole mouse embryos increased the survival of mice given 1025 R from 0 to 30%.³³

f. Consequences of Allogeneic or Xenogeneic Cell Transplantation

The most serious complication of allogeneic or xenogeneic bone marrow transplantation in irradiated animals is delayed mortality due to secondary disease.⁴² The first symptoms of secondary disease usually occur during the fourth or fifth week after transplantation and include primarily emaciation, diarrhea, and skin lesions (abrasions, ulcers, and localized loss of hair). The pathological changes of secondary disease include colitis, emaciation, dermatitis, extensive atrophy of lymphatic tissues, a high incidence of local inflammatory processes, and late hepatic necrosis. Two hypotheses have been proposed. One hypothesis suggests that as the graft matures, it sets up an immunological response against the antigens of the host, whereas another suggests that when the tissues of recipients recover, they react against the graft. A hypothesis suggests that the presence of donor tissue-associated antigen-presenting cells is a major factor in tissue rejection.⁹⁵

g. Radiation Chimera

When an animal is carrying cells of two genotypes, it is referred to as a chimera. If the above condition has been produced by irradiation, it is called a radiation chimera.

VI. MODIFICATION OF GI SYNDROMES

Many agents and procedures, when used before or after X-irradiation, modify the radiation response of the small intestine. Some of them are described below.

A. RADIOPROTECTORS

β -Mercaptoethylamine (MEA), when given before whole-body irradiation, reduces damage to the alimentary tract.³³ MEA (100 mg/kg), given 15 min before exposure of exteriorized segments of the small intestine, increases the ${}^{15}\text{LD}_{100}$ value from 1600 to 2000 R, the DRF being 1.26.⁶⁶ A mixture of chemical protectors offers better protection to the duodenal stem cells of mice than a single administration of any of the most potent protectors.^{67,68}

B. RADIOTHERAPEUTIC AGENTS AND PROCEDURES

1. Clamping of Superior Mesenteric Vessels

Clamping of the superior mesenteric vessels during irradiation of the small intestine of the rat with X-ray exposures below 3000 R reduces mortality, body weight loss, and intestine weight loss.⁶⁹ It raises the $^{15}\text{LD}_{100}$ value from 1600 to 3500 R, the DRF being about 2.3. The above procedure obviously produces circulatory hypoxia in the small intestine, and the reduced amount of oxygen during irradiation may be one of the important factors in the mechanism of radiation protection.

2. Combined Treatment of Radioprotectors and Mesenteric Vessel Clamping

The combination of MEA (100 mg/kg, i.p., 15 min before exposure) and the clamping of mesenteric vessels during exposure produces a DRF of about 2.8.⁶⁶ It appears that MEA and clamping may have, at least in part, different mechanisms of radiation protection.

3. Significance of Bile

The presence of bile in the intestinal lumen influences the characteristics of the postirradiation intestinal syndrome. Ligation of the bile duct immediately before X-irradiation (1500 R, whole-body) increased the average mean survival time in the rat from 3.3 to 5.6 days.⁷⁰ When the bile duct was ligated 1 day after exposure, the mean survival time was increased from 3.3 to 5.0 days.⁷⁰ The loss of sodium due to net leakage across the intestinal wall is of minor importance in the radiation syndrome. The loss of sodium via bile is an important factor in the cause of death.⁷⁰ Bile removes the mucous from the intestinal epithelium of irradiated rats. Diarrhea following intestinal irradiation is a loss of mucous as well as of water and salt; when the loss of mucous is prevented by the diversion of bile away from the intestinal lumen, diarrhea does not occur, even though the basic radiation damage to the cells of the intestinal epithelium is unaltered.⁷¹ It has been suggested that mucous may be part of the permeability barrier, which normally prevents loss of water and salts into the intestinal lumen from the intestinal tissues. The presence of bile does not influence the cell renewal rate of the rat small intestine.⁷²

4. Significance of Intestinal Flora

Intestinal flora appear to influence the severity of the intestinal syndrome. When germ-free mice are exposed to 1000 to 3000 R, they live twice as long as conventional mice given the same exposures.⁷³ The cell renewal rate of the intestinal mucosa of the germ-free mouse is lower than that in the conventional mouse.⁷⁴ The increased survival time in germ-free mice after whole-body exposure (3000 R) is ascribed to the longer life span of the mucosal cell. The migration of epithelial cells from the crypt to the villus tip takes 4.3 days in germ-free mice and 2.1 days in conventional mice. After 3000 R, the intestine is denuded in 3.5 days in conventional mice and not until 7 days in germ-free mice. Death occurs very soon after denudation in both groups.⁷⁵

Striking changes occurred in mid-small-intestinal and cecal flora of rats after a whole-body exposure of 1400 R or intestinal exposure of 2000 R. The changes reached a maximum 3 days after exposure. A consistent 100- to 10,000-fold increase in coliforms and enterococci occurred at both sites. Other organisms participated less constantly in the overgrowth. Massive bacterial invasion of the severely injured small-intestinal mucosa did not occur. There was no difference in bacterial invasion of mesenteric lymph nodes, spleen, liver, blood, or heart between control and irradiated animals. Thus, massive intestinal flora changes occur in the rat during the intestinal syndrome, but these changes are probably not significant in causing the animal's death.

5. Partial Gut Shielding and Surgical Removal of Irradiated Intestine

When a section of duodenum or ileum approximately 6 cm long was shielded, mortality decreased from 80 to 33% after 1000 R.⁷⁷ Surgical removal of the irradiated intestines in the animals increased the survival by about 33%.¹³ When 70% of the ileum and the jejunum were removed before X-irradiation, the resected animals were less sensitive to irradiation than were sham-resected animals.⁷⁸

6. Effect of Chilling

Chilling of the small intestine of the rat immediately after X-irradiation (750 R, whole-body) postponed depression of the mitotic count and degeneration of the crypt cells.⁷⁹ Mitotic depression and cellular degeneration occurred, however, when the tissue was rewarmed.

7. Use of Antibiotic and Electrolytes

Dogs exposed to 1300–1800 R died within 2.6–4.3 days, with an average survival time of 3.5 days. When experimental therapy was carried out using parenteral infusions of balanced electrolyte solutions, plasma, protein hydrolysates, antibiotics, and vitamin supplements, the survival time was increased about 7.2 days.⁸⁰ The administration of a sterile 0.9% solution containing either sodium chloride or a mixture of sodium chloride, sodium acetate, and potassium chloride during the critical 2- to 5-day period after irradiation (1500 R) had no therapeutic value. However, treatment with an antibiotic during the same period increased the survival time from 3.8 to 5.8 days. When the antibiotic was combined with an infusion solution, the average survival time increased to 8.2 days.⁸¹

VII. TREATMENT OF ACCIDENTALLY EXPOSED INDIVIDUALS

The pathogenesis of the radiation syndrome is better understood than that of many clinical conditions that are dealt with effectively in the course of medical practice.⁸² The therapeutic principles involved in the radiation syndrome are based on the knowledge of pathological changes; as with other disorders, the type of therapy and its time of administration depend on a complete and continuing clinical appraisal of findings in the individual patient.⁸² Bond et al.⁸² published an excellent account of therapy of the heavily exposed individual.

VIII. CLASSIFICATION OF PATIENTS WITH ACUTE RADIATION SYNDROMES

A. BASIS FOR PATIENT CLASSIFICATION

It has been suggested⁸² that therapy should not be based on the radiation dose. Therapy should be guided only by the daily appraisal of clinical and laboratory findings; it should be given only when clearly indicated, not prophylactically.

B. PHYSICAL CONSIDERATIONS

1. Type of Radiation

It is necessary to establish whether the individual has been exposed to X-rays or neutrons. Neutron exposure may induce radioactivity in many parts of the body; therefore, the hair, nails, blood, etc. will emit radiation. If the neutron-induced radioactivity is detected in the body, such a person should be isolated from those who have been exposed to X- or gamma-radiation.

2. Dosimetry

An effort should be made to obtain as accurate a dose estimate as is possible, as early as possible; however, such dose estimates are of limited value in patient management for the following reasons:⁸²

1. Suitable dose-measuring devices may be absent when an accident occurs, and the retrospective dose evaluation usually is not completed until after the time of optimal usefulness. An early dose estimate may be in error by a considerable factor.
2. Two or more kinds of radiation usually are involved — the relative effectiveness and degree of additivity of which are not known.
3. Considerable disagreement on dose-effect relationships in man exists at present.
4. If the statistical dose-effect relationships were known for man, it would still be impossible to determine in advance the sensitivity of a single individual.
5. Geometry and shielding effects leading to gross inhomogeneities in the absorbed dose may be difficult or impossible to evaluate.

IX. MANAGEMENT OF THE INDIVIDUAL PATIENT

A. GENERAL CONSIDERATIONS

Radiation exposure should not be considered an extreme emergency. There is no justification for hasty therapeutic procedures without careful consideration.⁸² The most challenging therapeutic problem is related to depression of the bone marrow and its sequelae produced by pancytopenia.⁸² The need for therapy and the time at which it should be considered are related to the depletion of peripheral blood elements and to the time during which the depletion occurs. *Functional replacement therapy* is employed here to indicate the use of antibiotics to augment impaired defense mechanisms and of fresh platelets to control bleeding. *Tissue replacement* or *bone marrow therapy* indicates the use of bone marrow or fetal tissue for transplantation.

B. EFFICACY OF FUNCTIONAL REPLACEMENT THERAPY

The use of antibiotics is well established in clinical medicine. Antibiotics alone are markedly effective in heavily irradiated mice,⁸³ less so in rats⁸⁴ and dogs,⁸⁵ and should be given whenever indicated. The value of fresh platelet transfusions in clinical thrombopenias is well known, and this ability to stop radiation-induced bleeding has been demonstrated in dogs.⁸⁶ Lyophilized platelets are ineffective.

The efficacy of replacement therapy has not been determined in irradiated human beings. However, in the dog, a near LD₁₀₀ dose can be converted to an LD₀ dose by functional replacement therapy.⁸² Fresh whole blood (out of the body less than an hour) can be given if the hematocrit is below normal; platelet-rich plasma can be given if the hematocrit is normal. Platelets are transfused in an amount corresponding to the number present in 20–40% of the blood volume of the dog.

C. CONSIDERATION OF BONE MARROW TRANSPLANTATION

The transplantation of bone marrow in irradiated rodents is very effective. The transfusion is effective at dose levels above LD₁₀₀, but is less effective or even harmful at lower dose levels. The latter may be due to an incomplete destruction of the host's immunity and resultant host-donor reaction. With some allogeneic transfusions and with xenogeneic transfusions, although there may be an early protective effect, many animals later develop the "secondary disease" or "wasting disease." Although autologous marrow transfusions have been of value

in dogs, allogeneic transfusions have not been at all, or are rarely, successful.⁸⁷ The Yugoslav radiation casualties were treated in Paris with allogeneic marrow, and evidence has been presented that the marrow "took" and was instrumental in the survival of the patients.⁸⁸ Some have regarded the evidence for a "take" as inconclusive. The successful allogeneic marrow transfusions in leukemic children,⁸⁹ including the late development of secondary disease, has been reported. The secondary syndrome was not necessarily lethal; however, its development must be considered one of the serious hazards associated with bone marrow transfusions. An apparently successful allogeneic bone marrow transfusion in a patient whose marrow was rendered aplastic with a chemotherapeutic agent has been reported.⁹⁰

D. REMOVAL OF INTERNAL CONTAMINATION

If the skin becomes contaminated with radioactive material, it should be thoroughly washed as soon as possible in order to reduce the chances of the contamination reaching the interior of the body via the lungs, gut, or broken skin. Radioactive material that has entered the alimentary canal can irradiate the walls as it passes along; there is also the possibility of absorption. Therefore, it is worthwhile to reduce the contact time within the bowel by administering an emetic such as magnesium sulfate. The above procedures are nonspecific remedies which may be applied to any isotope.

Isotopic dilution or *metabolic blocking* techniques have been of value in the case of radioiodine, because ^{131}I is volatile and is found in abundance in the atmosphere after nuclear bomb explosions and certain reactor accidents. A dose of potassium iodine containing 200 mg of stable iodine will inhibit absorption of radioiodine by the thyroid. Given 2 hr after exposure to radioiodine, it will reduce uptake by the gland by 80%, 6 hr after, by about 30%.⁹¹ A single dose is probably all that is necessary after a short exposure; but if personnel continue to work in an atmosphere of radioiodine, as perhaps during rescue operations, the administration of stable iodine will need to be repeated at about 4- to 6-hr intervals while the exposure lasts.⁹¹

After ingestion of $^3\text{H}_2\text{O}$, most of the radioactivity from the body can be removed by excessive intake of cold H_2O . Tritium has an effective half-life in the body of about 12 days, and therapy can reduce this to a few days.⁹¹ If ^3H -thymidine, a specific precursor of DNA, instead of ^3H -water has been ingested, the above therapy procedure would be useless.

Chelating agents form inactive complexes with certain metal ions and allow rapid excretion. They are used in the treatment of poisoning with certain heavy metals — for example, lead. Chelating agents may be of some value in cases of internal contamination with the rare earths, yttrium, plutonium, and thorium, and the actinide metals. To be most effective, chelating agents must be administered during the first day or so after the radioisotope has entered the body, when it is still in the temporary depots of the circulation and soft tissues. Chelating agents do not affect the permanent pool of the metal. Chelating agents by themselves are toxic, and this has limited their more frequent use.

X. THE CHERNOBYL EXPERIENCE

On April 26, 1986, the world's most serious nuclear accident occurred at the Chernobyl nuclear power station in the former Soviet Union, releasing excessive amounts of radioactive substances into the atmosphere.^{93,94} This accident raised important issues on managing the care and treatment of persons exposed to high doses of radiation. This issue has been discussed in a recent book.⁹² The medical response involved five phases: assessment, containment, reduction of radiation exposure to accident victims, estimation of exposure to individuals, and therapeutic treatment of those injured.

A. ASSESSMENT, CONTAINMENT, AND EVACUATION

Assessments were made on important parameters such as the amount and type of radioactivity released. Measures to contain the spread of radioactivity that resulted from the accident were carried out. To limit the exposure to individuals located near the reactor, massive evacuations were conducted. Within 36 hours of the accident, 45,000 persons were evacuated inside of a distance of 4 km kilometers from the reactor. Two weeks after the accident, 90,000 additional individuals were evacuated from a 30-km radius.^{4,13,15}

B. DOSIMETRY

Physical dosimetry, such as the use of individual radiation meters or film badges, was of limited value at Chernobyl, because the monitoring devices were either unrecoverable or destroyed by the high levels of radiation involved. Biological dosimetry proved more effective at providing information on exposure levels, due to the limitations of physical dosimetry, but required a demanding amount of medical and technical expertise and resources. Furthermore, there were limitations to the accuracy of the biological dosimeters because of the thermal and chemical injuries that have an effect on important biological parameters.⁹⁴

C. TREATMENT

About 200 people were exposed to large doses of whole-body radiation. One hundred and five received about ≥ 1 – 2 Gy; 33 received less than 6 Gy, and 10 received ≥ 6 Gy. Thirteen persons were exposed to 560–1340 rads (5.6 to 13.4 Gy), which were in the range of GI syndrome.⁹⁶ Two transplant recipients who received an estimated dose of 5.6 and 8.7 Gy were alive more than 3 years after the accident. The others died due to various causes, including burns, intestinal pneumonitis, graft-vs.-host reaction, renal failure, and respiratory distress. In another six instances fetal liver cells — which, during the second trimester of gestation, are a good source of hematopoietic stem cells — were transplanted. In theory, the fetal liver cells are less likely to have host-vs.-graft reactions than cells in marrow.⁹³ During the 3 months following the Chernobyl accident, 11 of 13 patients who had received bone marrow transplants and all of those who had received fetal liver cell transplants died. Eventually, the two persons who survived a 3-year follow-up for bone marrow transplant rejected the graft and died. The transplants may not have led to the survival of any victims, because those persons who received the transplants showed extensive host-vs.-graft reaction. These results indicate that the risk of graft-vs.-host disease must be considered when the benefits of this treatment are being considered.

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Chapter 9

RADIATION DAMAGE OF SKIN AND MUCOUS MEMBRANE

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I. INTRODUCTION

Extensive works have been published on the effect of ionizing radiation on the skin. In fact, the first effect of ionizing radiation appeared as an acute radiation dermatitis, or so-called "radium burn," among investigators working with radium. Prior to the introduction of "R" as a measurement of radiation exposure, radiation therapists utilized skin reaction — which they referred to as the *threshold erythema dose* (TED) — as a measurement of radiation dose.

II. THE SKIN

The degree of skin lesions after irradiation depends on several factors: (1) radiation dose, (2) quality of radiation, (3) time over which the dose is administered, (4) size of the field, and (5) anatomical location. The radiosensitivity of the skin of the same anatomical location markedly differs from one individual to another. Furthermore, the various structures of the skin display marked differences in radiosensitivity. The extent and rate of recovery depend upon the degree of injuries.

A. RELATIVE RADIOSENSITIVITY OF VARIOUS STRUCTURES

The relative radiosensitivity of various structures of the skin varies markedly. The hair follicles are highly radiosensitive (Table 9.1).⁴ The hairs of the scalp of both sexes and the beard in men are very radiosensitive. The radiosensitivity of hair follicles appears to be linked with the rate of hair growth. The more active the growth of hair, the more radiosensitive the hair follicle will be. Thus, the hair follicles of children, in comparable areas of hair growth, are generally more radiosensitive than those of adults. When the regrowth of hairs occurs after

depilation, the color of the new hairs in the human does not generally change to a significant degree. Occasionally, the new hairs may be gray, and the physical characteristics of the hair may change — i.e., curly hair may become straight. Unlike humans, lower mammals always show graying of hairs after irradiation.

The radiosensitivity of the sebaceous glands is similar to that of the hair follicles, but the damage tends to persist longer than that in hair follicles (Table 9.1). Sweat glands are not as radiosensitive as the hair follicles or sebaceous glands. Degenerative changes in the sebaceous glands are largely responsible for the dryness and the tendency toward scaling of irradiated skin.

B. RADIATION DERMATITIS

The radiation responses of skin are referred to as radiation dermatitis. The intensity of radiation dermatitis varies, depending upon the radiation factors used. The various phases of radiation dermatitis are discussed here under the following arbitrary conditions for radiation therapy.¹⁰ These include 250-kVp X-irradiation, half-value layer (HVL) 1.0 mm copper, field size 15 × 15 cm, daily skin dose 200 rads to a total skin dose of 4000 rads in 4 weeks or ⁶⁰Co-irradiation with bolus, daily skin dose of 250 rads, and a maximal skin dose of 5000 rads in 4 weeks.

Rubin and Casarett¹⁰ have divided the skin response during and after radiation therapy into the following categories: (1) acute clinical period, (2) subacute clinical period, (3) chronic clinical period, and (4) late clinical period.

1. Acute Clinical Periods (Within 6 Months)

a. *Initial Erythema*

The initial erythema is generally seen within a few hours to a few days and lasts only a day or so. This type of skin reaction is largely due to capillary dilation caused by the release of histamine-like substances.

b. *Dry Desquamation*

This condition gradually develops after initial erythema. The dry desquamation is characterized by atrophy of epidermal papillae, epidermal hypoplasia, and vascular changes. If the radiation dose is limited to 3000 rads in 3 weeks, dry desquamation is generally accompanied by temporary depilation and incomplete sloughing of the epidermis.¹⁰

c. *Erythema Proper*

The erythema proper generally develops in the third or fourth week. The skin becomes red, warm, edematous, and tender, and it exhibits a burning sensation. The erythema proper appears to be associated with obstructive changes in arterioles.

d. *Moist Desquamation (Exudative Radiation Dermatitis)*

If the skin reaction during the period of erythema proper is severe, acute radiation dermatitis may develop.¹⁰ With moderately large radiation doses (4000 rads or more in 4 weeks, or 2000 rads in a single dose), moist desquamation may occur at the fourth week. Some of the pathological changes include the following: (1) blister formation in the epidermis, (2) dermal hypoplasia, (3) edema, (4) inflammatory cell infiltration, (5) damage of vascular and connective tissue, and (6) permanent depilation.¹⁰

e. *Recovery of Skin*

The recovery of skin depends upon two factors: (1) usual therapeutic doses have not been exceeded, and (2) vascular and connective tissue of the dermis have not been severely damaged.¹⁰ Reepithelialization begins with the island of cells that generally coalesces in 6–8 weeks. The new skin is thin and pink, but it returns to normal appearance in 2–3 months.

TABLE 9.1
Changes in Mouse Skin as a Function of Exposure

Single Exposure (R)	Type of Tissue	Effect
100-200	Hair follicles	Transient mitotic delay, degeneration of germinal cells
400-500	Hair follicles	Temporary depilation (loss of hair) in about 3 weeks resulting from damage to germinal cells; depilation lasts only for a month or two
700 or more	Hair follicles	Depilation is permanent
700 or more	Sebaceous glands	A reduction in the secretion begins in a week; after a month, the few surviving cells often show degenerative changes
400-500	Sweat glands	Temporary decrease or cessation of sweat secretion; epithelial cells show considerable degenerative changes in the first few days; damage persists longer than hair follicles or sebaceous gland

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 241. With permission.

f. Development of Necrosis

Necrosis of the skin after irradiation seldom occurs; however, infection may cause necrosis of the irradiated area.¹⁰ Even without infection, if the blood vessels and connective tissues are damaged due to radiation dose or preexisting disease, a necrotic ulcer may develop in about 2 months.¹⁰

g. Hyperpigmentation

Following erythema proper, an increase in pigmentation, which is primarily due to an increase in the synthesis of melanin, may occur. The degree of pigmentation varies from one region of the skin to another in the same individual, and from one individual to another.

2. Subacute Clinical Period (6 Months to 1 Year)

Signs of atrophy may appear. Pigmentation may fade and vitiligo may become established, particularly in black patients. Ulcerations may appear in an area of re-epithelialization.¹⁰

3. Chronic Clinical Period (1 to 5 Years)

During this period, atrophy, ulceration, and deep fibrosis may be observed.¹⁰ The skin, after chronic exposure, may show a sign of epidermal hyperplasia and hyperkeratosis.¹⁰ The epidermis may become either hypertrophic or atrophic. The hypertrophic skin exhibits hyperkeratosis and marked keratinization of lesions. In addition, the epidermis is thicker than normal, the folds are exaggerated, and there is partial or complete depilation.¹⁰ The atrophic skin shows the thinner, smooth, and scaly epidermis, which is susceptible to even minor trauma or infection that can lead to necrosis. Ulceration, once formed, is difficult to heal because of poor vascularization and damaged connective tissue.

4. Late Clinical Period (More Than 5 Years)

Chronic radiation dermatitis may appear and is associated with an increase in the incidence of skin neoplasms, primarily squamous cell carcinoma.

C. VARIABLE FACTORS IN THE RADIATION RESPONSE OF THE SKIN

1. Physical Factors

a. Total Dose

The degree of erythema and the severity of skin reaction are proportional to the total dose. A dose of 3000 rads (fractionated dose) results in a dry desquamation, whereas a dose of 4000

rads (fractionated dose) produces a moist desquamation. Doses of greater magnitude may produce severe necrosis.

b. Dose Rate

The higher the dose rate, the greater the skin reaction will be.

c. Fractionation

A fractionated dose is less effective than a single dose in producing skin erythema. The relative effectiveness of a fractionated dose is dependent on the time intervals between fractions and the size of the fractions. A longer time interval and smaller fraction size would produce relatively less severe skin reaction.

d. Different Qualities of Radiation

The threshold erythema dose (TED) varies markedly as a function of the quality of the beam, which is often expressed in terms of the half-value layer (HVL) of Cu or Al. Radiation of lower energy will give a lower HVL. The TED varies by a factor of more than 3 from HVL of 1 mm Al (0.03 mm Cu) to 10 mm Cu.⁵ Electron beam therapy in the 1–3 MeV range limits skin reaction. For β radiation,¹⁰ threshold erythema requires 34 $\mu\text{Ci}/\text{hr}/\text{cm}^2$ (143 R), dry desquamation requires about 2000 $\mu\text{Ci}/\text{hr}/\text{cm}^2$ (1700 R), and bulbous epidermatitis needs about 4400 $\mu\text{Ci}/\text{hr}/\text{cm}^2$ (7200 R). The RBE of cyclotron-accelerated α particles for acute skin reaction is about 3 ± 1.0 ; for survival of hair follicles, it is about 2.1 ± 0.7 . Irradiation up to 500 R over a period of 1 year produced graying of hair and depilation of animals.³ Small daily doses show evidence of a disproportionately high skin damage and reduced recovery.⁶

e. Tolerance

The tolerance of the skin varies with the size and volume of the area irradiated. The dose required to produce necrosis for a field size of 1 cm^2 is about 4800 R, whereas for a field size of 10 cm^2 , it is about 2500 R — a difference of about 2.¹

2. Biological Factors

a. Anatomical Location

Various sites have been listed in order of decreasing radiosensitivity by Kalz:⁹

1. Anterior aspects of neck
2. Flexor surface of the extremities, chest, and abdomen
3. Face
4. Back and extensor surfaces of the extremities
5. Neck
6. Scalp
7. Palms and soles

Areas of moisture and friction (such as the axilla, groin, and skin folds) are most sensitive. The difference in radiosensitivity may, in part, be due to differences in the cell kinetics of the epidermis and differences in epidermal thickness.

b. Skin Pigmentation

Although the pigmentation of the skin is of radioprotective value against UV radiation, it has no significant effect against ionizing radiation.⁷

c. Sex and Age

Radiosensitivity of skin is independent of sex, although some investigators suggest that females are more radiosensitive than males.¹⁰ Age is an important factor in skin reaction.

Although the skin of children may be more sensitive than adult skin, it recovers much faster. Therefore, the moist desquamation frequently seen in adults is rarely seen in children.

d. Hormones

The response of patients with hyperthyroidism is more severe than in the euthyroid individual.¹⁰

e. Anemia and Anoxia

Severe anemia and poor vascularization decrease the skin reaction.¹⁰

3. Radiosensitivity of Normal vs. Grafted Skin

The radiosensitivity of grafted skin depends upon the age of the graft. The young graft (less than 3 months old) shows greater radiosensitivity than normal skin. A graft of intermediate age (3–12 months) shows radiation responses similar to those of normal skin. An old graft (older than 1 year) generally shows little reaction to irradiation; once it does, it does not recover and leads to necrosis.

4. Radiation Response of Animal Skin

Epithelial changes in the skin of the mouse are observed after a dose as low as 35 R. The number of mitotic cells in the epidermis substantially decreases.⁴ Doses in excess of 2700 R (single dose) are necessary to produce erythema in rabbit skin.⁴ A dose of 4000 R from ³²P causes denudation of the mouse epidermis. The threshold dose for moist desquamation at a dose rate of 600 R/day was 6000 R (⁹⁰Sr–⁹⁰Y) in female rats. Similar changes have been observed in other species. The threshold dose for the inhibition of mitosis in guinea pig skin with X-rays, or beta- or gamma-irradiation, is about 400 R.⁴ The RBE of thermal neutrons for the inhibition of mitosis in mouse skin is about 1.7.

In rabbits, total-body X-irradiation with 600 and 1200 R causes a decrease in skin DNA and RNA synthesis. An exposure of 700 R causes a substantial reduction in the hexokinase activity of skin, and even 3 weeks after exposure the enzyme activity remains 25–500% below the normal value.⁴

A small increase in the total acid-soluble and inorganic phosphorus of the skin was followed by a 20–30% decrease in the total acid-soluble phosphorus. Phosphagen phosphorus and ATP levels decrease for a few days, then rise to about 50% above normal values.

In laboratory animals, doses in excess of 2000 R are generally required to produce depilation, whereas in humans only 700 R is needed to produce the same effect. In the C57BL mouse strain, a whole-body exposure (1000–1500 R, 100 kV) is required for permanent depilation.⁴ The matrix cells of the growing hair follicles are the most radiosensitive cells in the skin.⁴ The regrown hairs, after a temporary depilation, often are not accompanied by sebaceous glands, and therefore are coarse.⁴ The new hairs may be white or gray, owing to a lack of pigmentation. Graying of hair in mice and hamster may be seen after an exposure of 200 R.

III. THE MUCOUS MEMBRANES

A. RADIATION RESPONSE

The radiation-induced changes of mucous membranes (radiation mucositis) are similar to those observed in the skin.¹⁰ However, the responses appear somewhat earlier in the mucosa than in the skin, and the rates of recovery may be more rapid. Like skin, the degrees of radiation damage and recovery in the oral mucosa depend upon the size of the dose, the quality of radiation, the temporal aspects, mode of dose administration, and other biological factors.

The changes in the mucosa are described under a standard situation.¹⁰ It is presumed that an individual of average radioresponsiveness receives a course of radiation through 8×10 cm fields cross-firing the oropharynx with the following set of factors: (1) 280 kV, HVL 1.0 mm Cu, irradiation at a daily dose of 200 R and a weekly dose of 1000 R to a total dose of 5000 R or ^{60}Co , HVL 12.0 mm Cu; (2) irradiation at a daily dose of 200 R and a weekly dose of 1200 R to a total dose of 6000 R.

Rubin and Casarett¹⁰ have divided the response of the mucous membrane during and after radiation therapy into the following categories: (1) acute clinical period, (2) subacute clinical period, (3) chronic clinical period, and (4) late clinical period.

1. Acute Clinical Period (Within 6 Months)

Radiomucositis is noted before the onset of radiation dermatitis during treatment of head and neck cancer.¹⁰

At the end of the second week (2000–2400 R), the patient complains of soreness, dysphagia, and pain when swallowing.¹⁰ Dryness of the mouth may be observed, and coarse and highly seasoned foods are poorly tolerated. Taste may be altered and appetite may be decreased. Mastication of dry, solid food is more difficult. Erythema, prominence of papillae, and patchy radiation mucositis limited to the palate and uvula may appear. There may also be hypersecretion by the mucous glands.¹⁰

At the end of the third week (3000 to 3600 R), the throat may become congested and the tongue swollen.¹⁰ The saliva appears thick and tenacious. The patchy mucositis becomes more confluent and extends onto the tonsilar pillars.¹⁰

At the end of the fourth week (4000–4800 R), the degree of damage increases. The mucositis extends onto the buccal mucosa, and the epithelial surface is denuded.

At the end of the fifth week (5000–6000 R), the damage to the mucosa is at a maximal level, and maintenance of nutrition becomes very difficult. The mucositis is manifested by a whitish to yellowish pseudomembrane, which is confluent and limited to the irradiated area.¹⁰ It is difficult to remove with scraping. If this pseudomembrane is removed, a superficial ulceration which bleeds can be observed. The mucositis covers all the tissues of the oropharynx including the tongue, which is the last to show the radiation response.¹⁰

Regeneration of mucosa begins after the completion of therapy. This may be completed in about 1 or 2 months after irradiation.¹⁰ At the end of radiation therapy, varying degrees of squamous cell metaplasia and hyperplasia may be seen in the mucous glands of the oral mucosa.¹⁰

2. Subacute Clinical Period (6 Months to 1 Year)

During this period, the regeneration of mucosa may be incomplete. Like skin, the mucosa may exhibit progressive fibrosis and may become more susceptible to trauma and infection than normal mucosa. Consequently, ulceration may sometimes develop.¹⁰

3. Chronic Clinical Period (1 to 5 Years)

During this period, the mucosal damage increases. Breakdown and ulceration of the mucosa are more frequent during this period than during the subacute period, and they may develop suddenly in some cases.¹⁰ The supporting tissue underlying the ulcerated regions may reveal substantial scarring, reduction of fine vasculature, and occlusion of small arteries. Trauma and infection often precipitate ulcerations.

4. Late Clinical Period (5 Years or More)

During this period, soreness and pain in the irradiated area of oral cavities may develop. In the upper alimentary tract, dysphagia is a common indicator of new lesions. Neoplasms of the laryngopharynx following radiation therapy have been reported.^{2,8}

B. VARIABLE FACTORS IN THE RADIATION RESPONSE OF MUCOSA**1. Physical Factors**

If radiation exposures (3000–4000 R) are delivered in 14 days, the radiation mucositis appears in 14 days, subsides in 28 days, and is followed by radiation dermatitis. If the same dose is delivered in 10 days, the mucositis is earlier in onset and later in healing, and overlaps radiation dermatitis. If the same dose is delivered in 18 days, the mucositis appears later and is separated from radiation dermatitis by 1 week.¹⁰

a. Volume

The degree of mucosal damage is proportional to the field size irradiated.

2. Biological Factors**a. Anatomical Sites**

The anatomical sites, in order of the appearance of radiation mucositis, are the following:

1. The soft palate and its pillars
2. The posterior wall of the pharynx
3. The tonsilar regions
4. The floor of the mouth
5. The anterior of the buccal surface
6. The mucosa of the lower alveolus
7. The laryngeal face of the epiglottis
8. The laryngeal surface of the tongue
9. The vocal cord
10. The inferior surface of the tongue¹⁰

The recovery in mucosa occurs in a manner similar to that observed in skin. The same dose schedules are more easily tolerated by children than adults. The mucosa of children is more sensitive than the mucosa of adults, but it also recovers more rapidly.¹⁰

IV. SUMMARY AND COMMENTS

The degree of radiation response of the skin and mucous membrane after irradiation depends on several factors: (1) radiation dose, (2) quality of radiation, (3) dose rate, (4) size of the field, and (5) anatomical location. According to these variables, the extent of damage may range from minimal degenerative changes to total necrosis. Depending upon the extent of injury, the recovery may be rapid and complete or may not occur at all.

Skin changes after radiation may be characterized by the appearance of initial erythema, dry desquamation, erythema proper, moist desquamation, and necrosis. Hyperpigmentation or depigmentation may occur in some individuals. Neoplasms of the skin may develop as a late effect. The radiosensitivity of grafted skin depends upon the age of the graft. The young graft (less than 3 months old) shows greater radiosensitivity than normal skin. The graft of intermediate age (3–12 months old) shows greater radiosensitivity than normal skin. The graft of intermediate age (3–12 months) shows radiation responses similar to those of normal skin. The old graft (more than 1 year) shows little reaction to irradiation; once it does, it does not recover and leads to necrosis.

Changes in the mucous membrane after irradiation are characterized by the development of radiation mucositis, which generally appears earlier than radiation dermatitis. The mucositis initially appears as patchy and later spreads over the entire irradiated area. At the end of radiation therapy, varying degrees of squamous cell metaplasia and hyperplasia may be seen.

Occasionally, ulceration may be observed. Neoplasms of the laryngopharynx following irradiation may develop as a late effect.

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Chapter 10

RADIATION DAMAGE OF THE NERVOUS SYSTEM

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I. INTRODUCTION

It is generally assumed that adult nervous tissue is highly radioresistant. This may be true when based on the criterion of morphological damages that are manifested within a few months. Functional changes in the neurons occur at comparatively low doses. The development of radiation-induced changes in the brain during and after irradiation depends upon several physical and biological factors.

II. RELATIVE RADIOSENSITIVITY OF CELLS

During development, some of the cells lining the inner layer of the neural tube differentiate into neuroblasts, which do not divide. Each neuroblast matures into a neuron. Some cells of

neural tubes differentiate into spongioblasts, which give rise to supporting elements of the nervous system. The neuroblasts are most radiosensitive on the criterion of cell death; even a few rads can damage neuroblasts. Significant loss of neuroblasts could lead to severe brain abnormalities. The changes in the cellular elements of the adult nervous system are described below.

A. NEURONS

The neuron is subdivided according to anatomical and functional criteria. The perikaryon includes and surrounds the nucleus, and contains most of the cytoplasmic organelles. The dendrites and axons arise from the perikaryon. At its end, the axon splits into branches, each forming a presynaptic terminal. The various parts of a neuron show differential radiation response.

1. Perikaryon

The death of neurons is primarily due to cytoplasmic damage. Studies with microbeams have shown that neurons with very small perikaryons, such as granule cells, are more sensitive than neurons with large perikaryons — at least on the criterion of cell death. Two hours after the mouse cerebellum was irradiated (60,000 rads) with a microbeam, the granule cells contained damaged mitochondria, broken cell membranes, and serrated nuclear membranes, whereas Purkinje cells were normal.¹ It has been proposed that this difference is due to a better repair capacity of cells with large perikaryons.² One study has shown that a loss of isolated ventral horn cells occurs in parabiotic rats 1 year after whole-body X-irradiation.³

2. Dendrites

These are very radioresistant. In laminar lesions produced by 33,000 rads of 10-MeV protons, it was found⁴ that the exposed top dendrites of nonirradiated perikaryons were not damaged.⁴ However, a reduction in the degree of dendritic arborization was observed 4 weeks after facial exposure of monkey cortex with 3500 R of X-rays.⁵ The damage to dendrites may not reflect a direct effect of irradiation on dendrites, but rather a response of the entire neuron to irradiation.

3. Axons

Axons are metabolically inactive and are also highly radioresistant on the criterion of structural changes. This view is supported by an electron microscopic study on tissue culture,⁶ on sympathetic peripheral nerves irradiated *in situ*,⁷ and on the brains of experimental animals.¹ This may not apply to immature axons. Honeycombed structures were present in cerebral axons of 30-day-old rats exposed to 1000 R on the second day of life.⁸ An abundant and overshooting regrowth of axons was observed a few weeks following their acute destruction. Mouse cerebellum irradiated with 100,000 rads of 20-MeV deuterons showed an increased number of poorly aligned axons, which traverse the lesion 2 years after exposure.⁹ An acute demyelination was observed in the rat brain 96 hr after irradiation with 30,000 rads of 48-MeV α particles, whereas the axon remained preserved.¹⁰ Remyelination of the denuded fibers and of newly sprouted axons commenced about the 16th day of postirradiation and was complete at 4–7 months.

4. Synapses

Neurophysiological studies with intracellular electrodes showed that irradiation with 600 rads produced significant changes in monosynaptic excitatory and postsynaptic potentials.¹¹ Doses of 200 rads of whole-body irradiation caused changes in polysynaptic systems;¹² even lower doses resulted in an arousal response, implicating the reticular activating system.¹³

These changes were not correlated with structural changes. The preservation of synapses was observed after 66,000 rads of deuteron irradiation of the mouse cerebellum.¹ The precise mechanism of the radiation effect on synapses is unknown. It has been suggested¹⁴ that temporary changes in membrane permeability for potassium and sodium, liberation of pharmacologically active substances like serotonin and acetylcholine, and interaction between lipoproteins and free radicals may contribute to the synaptic changes after X-irradiation. In general, low doses of radiation resulted in a dose-rate-dependent stimulation of respiration, synthesis of acetylcholine, and cellular uptake of 5-hydroxytryptamine. Doses above 500 rads reduced metabolic activity.²

B. GLIAL CELLS

In the adult CNS, glial cells normally divide infrequently, but may proliferate rapidly following a proper stimulus such as tissue damage. These cells are relatively radioresistant. Astrocytes are among the most reactive elements in the nervous tissue of various species and show an accumulation of glycogen within 24 hr following irradiation of only 500 rads.¹⁵ Because myelin sheaths represent the redundant plasma membrane of oligodendrocytes, any large damage to these cells by radiation will produce demyelination. Oligodendrocytes function as satellite cells for neuronal perikaryons, which may undergo necrosis if the satellite cells are damaged.

Schwann cells, the sheath cells of the peripheral nervous system, are more radioresistant than their central counterparts. The reasons are unknown. Microgliaocytes, after irradiation, may show hypertrophy and, occasionally, proliferation.

C. BLOOD VESSELS AND BLOOD-BRAIN BARRIER

The present concept suggests that the blood-brain barrier is referred to as the absence of a perivascular space. The astrocytic basal lamina is fused with the basement membrane of the endothelial cell, and the barrier function is probably shared by the astrocytes. Thus, radiation damage of the blood-brain barrier may be associated with the delayed necrosis of the CNS.⁹ Whether the acute and delayed breakdown in blood-brain barrier function is responsible for tissue necrosis is not known. Most investigators believe that damage of the vascular system is the primary factor in the delayed necrosis of the brain.

D. WHITE MATTER VS. GRAY MATTER

The white matter of the CNS appears to be more sensitive to radionecrosis than the gray matter. In the brain, the brain stem seems to be the most radiosensitive area. Selective delayed necrosis of the paraventricular and supraoptic nuclei of the hypothalamus has been found.

The most prominent feature involved in the radionecrosis of the white matter is demyelination, which is due to the damage of oligodendrocytes. The degree of demyelination is variable. The delayed necrosis is considered secondary to the vascular damage. Therefore, the difference in radiosensitivity of white and gray matter may be due to the difference in vascularity. The gray matter has abundant blood vessels, whereas the white matter has poor blood vessel distribution. An abundance of blood vessels may provide protection against delayed necrosis.

III. RADIATION RESPONSE DURING RADIATION THERAPY

Rubin and Casarett¹⁶ divided the radiation response of the CNS into the following categories: (1) acute clinical period, (2) subacute clinical period, (3) chronic clinical period, and (4) late clinical period.

A. ACUTE CLINICAL PERIOD (WITHIN 6 MONTHS)

The brain receives high doses of radiation during treatment of brain tumors. The main sign and symptoms are a sudden increase in headache, lethargy, vomiting, and papilloedema. If irradiation is continued in spite of these symptoms, convulsive seizures may occur.

1. Effects on the Vascular System

The acute response of the CNS to irradiation is an acute inflammatory reaction which begins within hours or days after irradiation. The main features of the acute period are the following:

1. Damage to fine vasculature, especially to the endothelium
2. Impairment of capillary circulation
3. Increased capillary permeability
4. Plasmatic transudation
5. Pericapillary and interstitial edema and acute inflammation
6. Edematous loosening of perivascular astrocytes and nervous tissue, with damage to the blood-brain barrier
7. Some rupture of capillary walls with petechial hemorrhages
8. Vasculitis with spotty endothelial damage
9. Meningitis and choroid plexitis

At lower dose levels, these changes may be localized or transient, or they may be repaired. At higher dose levels, they may be more widespread, severe, and longer-lasting, and they may repair some early damage. However, permanent chronic damage may persist.

The composition of the perivascular and interstitial inflammatory cellular infiltrate may change from a predominance of polymorphonuclear cells (granulocytes) to a predominance of mononuclear cells (lymphoid and plasma cells) as a function of time after irradiation. There may be some connective tissue proliferation in the meninges and choroid plexus and in the walls of the arterioles and large vessels. The radiation-induced lesions of larger blood vessels require more time to express. Degeneration of vessel walls, occlusion of the lumen by thrombosis, and fibrosis may occur after larger radiation doses.

2. Effects on Neurons

The morphological changes in the neurons are not prominent during acute clinical periods; however, transient and reversible changes in cerebellar granule cells may be observed. In the monkey, an exposure of 3500 R produces no immediate morphological change in the neurons, but after 12 weeks some cellular disorganization and some pyknotic cells may be seen in the cortex. Some reduction in dendritic processes may be noted.

3. Effects on Oligodendrites

The changes in oligodendrocytes are seen soon after the onset of the acute inflammatory reaction. These cells show swelling followed by degeneration and necrosis of some of the oligodendrocytes. Demyelination may occur within a few weeks or months after irradiation. A single exposure of 4000 R to the monkey brain causes focal areas of demyelination in the white matter, primarily around obliterated capillaries at about 18 weeks after irradiation.

Demyelination occurs in the irradiated human brain and spinal cord. In three patients who received an exposure of 5500 R of ^{60}Co gamma-radiation in 1 month to tumors in the region of the middle ear or jugular bulb, neurological symptoms characteristic of acute disseminated sclerosis developed 10 weeks after irradiation.¹⁷ One patient died 4 weeks after the onset of symptoms; the other two showed complete recovery from the symptoms. Another patient with

basal carcinoma of the ear, who received 5760 R (288 R/day) of ^{60}Co gamma-radiation developed neurological signs of disseminated demyelination of the brain in about 3 months.¹⁸

B. SUBACUTE CLINICAL PERIOD (6 MONTHS TO 1 YEAR)

During this period, the patient may completely recover or may show signs and symptoms of neurological involvement, such as convulsions, ataxia, and lack of coordination.

The severity of early radionecrotic lesions may increase, or new necrotic lesions may appear. Sometimes there may not be visible changes in the nervous tissue, but slow degenerative changes may occur in the capillary bed, arterioles, and venules — which may eventually lead to the necrosis. In the monkey, a marked loss of neurons and their dendritic arborization occurs 47 weeks after irradiation of the brain with 3500 rads.¹⁹

C. CHRONIC CLINICAL PERIOD (1 TO 5 YEARS)

During this period, the progression of vascular lesions and the onset of neurological involvement are slower than in the subacute period. Radionecrosis may occur; it has been reported to occur as late as 15 years after the completion of radiation therapy. Cases of epileptic seizures and convulsions may appear 7 years after completion of therapy.¹⁶ Poor cerebration, mental confusion, visual disturbances, and progressive deterioration to a vegetative stage can occur during this period. The pattern of changes resembles premature aging. This suggests that acceleration of vascular damage may be the primary factor in brain abnormalities.¹⁶ In addition, there have been several cases of delayed "radiation myelitis" of the brain stem, in which the first neurological symptoms appeared 11–20 months after a therapeutic exposure of the brain stem to 4500–6050 R.²⁰

D. LATE CLINICAL PERIOD (MORE THAN 5 YEARS)

Malignant intracranial neoplasms following radiation therapy have been observed.²¹

IV. SPINAL CORD

Radiation myelitis of the spinal cord after irradiation has been observed. Serious damage to the spinal cord may lead to invalidism and death in cases in which the original malignant tumor has been brought under control. A patient with lymphoma, who had received radiation exposure of between 2500 and 5000 R, developed severe spinal cord damage 13 months after exposure. The spinal cord lesions include hyaline thickening of the blood vessels with obliterated lumen and dissolution of white matter. All experimental animals (rats and monkeys) that received a localized spinal cord irradiation (3500 rads) developed neurological abnormalities 3–9 months after irradiation.¹⁶ The neurological signs included lower-limb motor weakness, which rapidly leads to paralysis with involvement of the sphincter.

In one review²² it was reported that none of the patients who survived after irradiation of the spinal cord during treatment of malignant diseases developed myelitis. However, in another report, 1% of the treated patients (1500–2000 R) and 8% of the long-term survivors had developed myelitis in the thoracic spinal cord.²³ The limit of tolerance appears to increase with time and radiation fraction number. The incidence of myelitis was 12.5% in patients treated with 4000 rads in an average of 15 fractions over 3 weeks.²⁴ The total doses (6000 rads in 35 fractions over 7 weeks and 5000 rads in 55 fractions over 5 weeks), delivered in a larger number of fractions, would exclude the possibility of myelitis. Such high doses can be tolerated if given in increments of 200 rads or less. Short courses of a few large fractions appear to be dangerous. Therefore, it has been suggested²³ that 1300 rads in two fractions and 3000 rads in ten fractions would appear to be the safe limit.

The irradiation of the brain stem and cerebellum affect the brain stem's conditioned lumbar cord and the flexor, extensor, and monosynaptic reflexes.²⁵ The preconditioning extensor and flexor monosynaptic reflexes were either both inhibited or both facilitated. The conditioned reflex was affected inversely, i.e., the flexor tended to be facilitated while the extensor was inhibited. Irradiation also affects the balance of brain-stem-level interaction on the descending systems to the spinal cord.

V. PERIPHERAL NERVES

The peripheral nerves are highly radioresistant. A dose that produces delayed necrosis of the spinal cord has no effect on the peripheral nerves.⁹ Peripheral nerves other than olfactory, retinal, and cochlear elements are also highly radioresistant. Neurological abnormalities in the median and ulnar nerves have been observed 15 months to 11 years after irradiation with 2400 rads.²⁶ In spinal and sympathetic ganglia, accumulation of glycogen and degeneration of satellite cells may be found within days after irradiation with 18,000 rads of protons. These alterations were similar to those observed in the CNS after much smaller doses.⁹

VI. BIOCHEMICAL CHANGES AFTER IRRADIATION

The alkaline phosphatase activity in the mouse brain markedly reduced within a few days after about 1000 rads. The degree of inhibition was dose-dependent.²⁷ In addition, a rapid increase in RNA content and succinate dehydrogenase activity in the nerve cells of Deiter's nucleus of the rabbit was observed 24 hr after 2000–3000 rads.²⁹ A relatively small increase was observed in the number of interstitial cells that incorporate ³H-thymidine in the rat spinal cord.⁹ Irradiated astrocytes showed a marked accumulation of glycogen and mucopolysaccharides.¹⁵

The concentrations of neurotransmitters, such as acetylcholine and 5-hydroxytryptamine, undergo significant changes. Small doses of up to 500 rads increase the levels of these amines,³⁰ whereas higher doses decrease their levels.^{27,31}

VII. VARIABLE FACTORS IN THE RADIATION RESPONSE OF NERVE TISSUE

A. PHYSICAL FACTORS

1. Time-Dose Relationship for Brain Damage

It is difficult to define the limits of radiation tolerance for the brain in spite of extensive studies on this subject. Animal studies indicate that the threshold dose may be about 2000–4000 rads. Most of these studies have used single doses and, therefore, are not useful in estimating radiation damage during radiation therapy. It is estimated that radionecrosis of the brain may occur in patients with brain tumors who received exposure of 4500 R in 30 days or 9000 R in 80 days.³²

2. Time-Dose Relationship for Spinal Cord Damage

Time-dose studies for the spinal cord are few, but it is believed that the tolerance level is about 5000 rads.¹⁶ The number of fractions and the time are very important in causing myelitis of the spinal cord.²³ It is estimated that 1300 rads in two fractions and 3000 rads in ten fractions may be the safe limit.

3. Effects of LET

Irradiation with high-LET α -particle radiation results in selective demyelination of the rat brain followed by remyelination¹⁰ — an effect not produced by low-LET radiation. The RBE of the Bragg peak proton beam for delayed necrosis³³ of the monkey brain is about 1.5. The RBE of other high-LET radiations for brain damage has not been estimated.

An average dose of 285,000 rads of X-rays to the frog sciatic nerve promptly inactivated impulse transmission. The RBE values for suppressed neural activity depended on the LET. Radiations with high-LET values had high RBE values.³⁴ The frog sciatic nerve exposed to either 200-kV X-rays or 47.5-MeV protons exhibited bioelectric changes prior to loss of excitability. The conduction velocity decreased and the detection period (stimulus-response interval) increased after the nerves absorbed only one fourth of the inactivation dose.

4. Effects of Dose Rate

The higher the dose rate, the greater the damage of neurons will be. Acute necrosis of cerebellar granule cells in the mouse was produced when 10,000 rads of X-rays was delivered to the head at a rate of 1000 rads/min or more; whereas the same dose, when delivered at the rate of 100 rads/min, was not effective.²⁸ When monkeys' brains were irradiated with 20,000 rads of 1600-MeV protons at a rate of 200 to 600 rads/min, the animals survived for more than 1 year. However, when a dose of 15,000 rads was delivered at the rate of 5000–7000 rads/min, the animals died within 11 days.³³ In addition, there was a reduction of the average latency for radiation necrosis of the rat spinal cord from 200 to 90 days, if a dose of 3500 rads was delivered in 20 sec instead of 10 min.⁹

5. Volume

The volume of tissue irradiated is very important in considering a threshold dose for the brain and spinal cord. It has been observed that as the field size increases, the tolerance decreases. It has been suggested²⁰ that the threshold doses should be 4500 R in 17 days for small fields (less than 50 cm²) and 3500 R in 17 days for large fields (more than 100 cm²).

B. BIOLOGICAL FACTORS

1. Preexisting Disease

This factor plays an important role in the modification of radiation injury. The gliosis secondary to the tumor and to irradiation are indistinguishable. In elderly patients, the vascular changes of aging may be additive to radiation damage in producing delayed necrosis of the brain.

2. Age

Age is a very important factor in determining the radiosensitivity of nervous tissue. It has been established that the nervous system of children is much more radiosensitive than that of adults.

3. Arteriosclerosis

This condition may be a factor in reducing radiation tolerance in older people. It is possible that radiation-induced changes in vessels may be additive to preexisting aging-induced changes. It has been suggested³⁴ that hypertension in the patient may enhance radiation-induced vascular changes in the CNS.

VIII. EFFECTS OF RADIATION ON BEHAVIOR

Because learning — particularly complex learning — is mainly dependent on the integrity of the cerebral cortex in mammals, and because the cortex is radioresistant, it is not surprising

to find that learned behavior in the adult is not adversely affected by whole-body irradiation. It is possible to demonstrate changes in cortical electrical activity and in some cortical enzyme systems at whole-body doses below the $^{30}\text{LD}_{50}$, but these changes may not be sufficient to produce any behavioral disturbance. Improved performance after irradiation has been reported as often as decreased performance.³⁵

Although whole-body radiation exposure does not significantly change the mammal's capability of learning, it markedly affects the nonintellectual behavior.³⁵ Depression of total body activity in monkeys and of running-wheel activity in rats was observed after irradiation.³⁵ Decreased mating activity in male swine and rats following fetal gamma-irradiation was observed.³⁵ Monkeys receiving a lethal dose reveal a lower amount of visual exploration during the 5 weeks preceding death and a pronounced drop in visual curiosity in the last week of life, when compared with controls.³⁵

When rat fetuses were irradiated on the 11th, 13th, 15th, 17th, and 19th day of postconception with 300–600 R, and tested on a Lashley type III maze on their 50th day of life, the behavioral defects were found to be directly related to the dose, and were more severe for groups irradiated on the 13th day.³⁵ In another study, rats were irradiated during the final week of pregnancy using 30, 90, 180, and 360 R. The offspring of animals receiving 90 R or more were significantly poorer maze learners than control animals.³⁵ In another study,³⁶ relatively low doses (25 and 50 R) were associated with deficient maze learning when they were delivered at 2.5, 6.5, and 7.5 days of gestation. The age at the time of testing is important. Rats exposed to 25 R at 2.5 days of gestation showed a defect in learning behavior when tested at 400 days.

It has been reported³⁷ that, in rats, a fractionated dose of less than 25 R produces a condition in which the animals avoid saccharin. The abdomen appears to be more sensitive in producing saccharin aversion. Exposure to 54 and 108 R delivered to the abdomen produced a decrement in saccharin consumption, whereas radiation of the head, thorax, or pelvis required a dose of 250 R to produce the same effect.

IX. SUMMARY AND COMMENTS

Among various cellular elements of the nervous system, the neuroblasts are most radiosensitive, whereas highly differentiated neurons are radioresistant. The neuroglial cells of the mature CNS are also relatively radioresistant. The blood vessels and blood-brain barrier are damaged by radiation, which causes delayed necrosis of the brain. Acute response of the CNS to irradiation primarily involves an acute inflammatory reaction. The morphological changes in the neurons are not prominent during this period. The oligodendrocytes show swelling and degenerative changes soon after acute inflammatory reaction. Early demyelination lesions in the irradiated human brain and spinal cord are observed. During the subacute period, the patient may completely recover or may show signs and symptoms of neurological involvement, such as convulsion, ataxia, and uncoordination. During the chronic clinical period, delayed necrosis and demyelination may be observed. During the late clinical period, malignant intracranial neoplasms following radiation therapy are observed.

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Chapter 11

RADIATION DAMAGE OF THE REPRODUCTIVE ORGANS

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I. INTRODUCTION

The gonads are extremely radiosensitive. Although there are sufficient data available on the effect of ionizing radiation on the reproductive tracts of animals, data on human gonads, especially the testis, are limited since the testis are rarely irradiated in clinical settings. Most of the information on man is based on the few accidental radiation exposures and some clinical observations. Since the frequency of female genital-tract cancer is relatively high, considerable knowledge has been accumulated regarding the radiosensitivity of the female reproductive tract.

II. THE MALE REPRODUCTIVE TRACT

A. EFFECT ON TESTIS

The processes of the formation of sperm are referred to as spermatogenesis. The highly proliferating spermatogonia line the seminiferous tubules and give rise to primary and secondary spermatocytes, which then form spermatids. The spermatids do not divide. Each spermatid matures into one spermatozoon. On the criterion of cell death, spermatogonia are most radiosensitive, whereas spermatids and spermatozoa are most radioresistant, but the possibility of genetic damage is high for all cellular elements. The interstitial cells that produce male hormone are also highly radioresistant. Therefore, men exposed at a sterilizing dose (500–600 rads) of radiation retain their fertility and produce seminal and prostatic fluid without sperm for a while. After irradiation, the testis becomes smaller and softer, and may become sterile. However, no change in the beard, voice, or social behavior has been observed.

The degree of damage and the extent of recovery depend on total dose, dose rate, and mode of delivery. At high exposures no recovery occurs, and at low exposures the recovery of

spermatogenic elements is complete. It must be emphasized that the recovered germinal cells carry a considerable amount of genetic damage, some of which may be lost as a function of time. In the human testis exposed to 1200 R, increases in alkaline phosphatase and in the levels of phospholipid and glycogen were observed within 10 days of exposure.¹

1. Sterility

Radiation damage to the testis causes sterility. In the male, sterility is never observed immediately after exposure, because the spermatids and spermatozoa are highly radioresistant. The time between the appearance of new spermatogonial stem cells and the formation of spermatozoa and their extrusion into the tubule lumen is estimated to be about 64 days.² Table 11.1 shows the estimated doses that will produce sterility in man.³ Three persons were accidentally exposed to 12–190 R whole-body irradiation. The sperm counts in these individuals were normal at 10 days, were greatly reduced or zero in two patients at 7–10 months, and were normal at 20 months.

One patient was accidentally exposed to fast neutrons and γ -rays — the average dose is estimated to be equivalent to 390 R of 80 keV X-rays and 26.4 R of γ -rays. This individual showed a progressive reduction of sperm count, which reached zero between 80 days and 7 months after irradiation. The above change was associated with marked degeneration of the spermatogenic elements and seminiferous tubules.⁵ Sign of recovery of spermatogenesis was observed at 20 months, and sperm counts showed progressive recovery. This patient, after recovery, produced a normal infant. In the absence of any follow-up information, it is difficult to estimate the radiation hazards among such infants.

2. Effect on Accessory Organs

Studies of irradiation on male accessory organs are not well documented.

a. Prostate and Seminal Vesicles

These structures are radioresistant. Exposure of the prostate and seminal vesicles with high radiation doses (6000–7000 rads) during treatment of bladder carcinoma and prostate carcinoma did not produce prostatitis or acute swelling of the gland.

b. Penis and Urethra

Very little information on the radiosensitivity of these structures is available. However, they are considered radioresistant on the criterion of cell death. If the therapeutic doses are markedly increased, necrosis in the accessory glands may appear in 6 months to years. The necrosis occurs due to damage of the fine vascular system and connective tissue.

TABLE 11.1
Effect of Radiation Sterility in Humans

Sex	Exposure (R)	Sterility
Male	500–600	Permanent sterility
Male	250	Temporary sterility for 12 months
Female	320–625	Permanent sterility
Female	125–150	Amenorrhea in 50% of women
Female	170	Temporary sterility for 1–3 years

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 301. With permission.

TABLE 11.2
Effect of Radiation Factors on Sterility
in Male Animals

Animal	Mode of Delivery	Total Exposure (R)	Sterility
Mice	8 R/week	200	39%
Mice	33 R/week	200	None
Rats	Single exposure	800	Temporary
Rats	In 5 days	800	Permanent

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 304. With permission.

3. Radiosensitivity of Animal Testis

Reduction in the number of mouse spermatogonia can be observed with 3 rads of ^{60}Co γ -rays, 2 rads of 2.5-MeV neutrons, and 1.5 rads of 14.1-MeV neutrons. RBEs of 8.18 for 14.1-MeV neutrons and 3.03 for 2.5-MeV neutrons were found.⁷ The LD₅₀ for spermatogonia is about 22 R of gamma-radiation, and the RBE of neutrons on this criterion is about 2. Based on 50% survival of spermatogonia RBE, values of positive and negative ions were 2.3 and 3.7, respectively.⁸

For sterility, the mode of delivery of radiation is an important factor. The fractionated dose produces more severe sterility than does the single dose (Table 11.2). However, when the total dose (300 or 600 R) was given at 0.8 or 90 R/min, the animals regain fertility at about the same time, indicating that the killing of spermatogonia was unaffected by this dose rate range.⁷ Chronic irradiation is more effective in reducing initial spermatozoa output, but allows better recovery than the single dose.⁹ In another study¹⁰ it was found that the time intervals between fractionations and total dose were important in the persistence of sterility. The mean time needed until return of fertility was 81 ± 4.1 days after the exposure of the testis with a single dose of 600 R. When the time interval of fractionation was 144 hr, the period of return to fertility (83.8 ± 1.2 days) was not significantly different from those that were irradiated with a single dose. However, when the time interval was 24, 48, or 72 hr, the time until return to fertility was markedly delayed (100–111 days).

The abscopal effect of ionizing radiation has been demonstrated on the rate of semen production in the guinea pig.¹¹ There was no effect of head irradiation (300 R) on the sperm output; however, irradiation of the body (testes and head shielded) produced a depression in sperm output, but there was no additional depression (Table 11.3) produced by irradiation of the head and body (testes shielded). Thus, the abscopal effect of irradiation on sperm production is not due to a direct radiation effect on the hypothalamic hypophyseal structures.

Change in weight of the testis has been considered as a quantitative measure of radiation damage. This effect is an exponential function of dose. The weight loss is due to actual loss of cells, and correlates well with the histological features. The RBE of slow neutrons for weight loss is about 1.2 to 2.5.

Radioprotective agents such as dopamine,¹² when given immediately before whole-body exposure (1000 R), markedly increased the survival rate. The weights of the testes in those irradiated animals that survived by virtue of dopamine treatment continued to decline for 54 days, after which the weight leveled off (Figure 11.1).

The interstitial cells remained intact even after a dose of 5000 R.¹³

TABLE 11.3
**Effect of Irradiation (300 R) on Sperm Output Following
 Exposure of Various Areas of Guinea Pig**

Portion of Body Irradiated	Period of Decreased Sperm Output	Time Required to Obtain Normal Sperm Output
Testis (head and body shielded)	6-7 weeks	21 weeks
Whole-body	12 weeks	No significant recovery after 42 weeks
Head and body (testis shielded)	1 week	47 weeks
Head (testis and body shielded)	No effect	No effect
Body (testis and head shielded)	1 week	47 weeks

Note: The original sperm level of 9.8×10^6 cells per ejaculation fell to less than 1×10^6 cells per ejaculation.

Data are summarized from Freund, M. and Borrelli, F.J., *Radiat. Res.*, 24, 67, 1965.

4. Effect on Sterility in Young Animals

Both sexes are most radiosensitive during the period about 7-21 days after birth, on the criterion of sterility. An exposure of 100 R sterilizes young female mice immediately, completely, and irreversibly. An exposure of 100 R does not sterilize the young male, although embryos from such males gave a high percentage of nonviable offspring.¹⁴

When C57 mice were exposed to a low dose rate of gamma-radiation (2, 5, 10, and 20 R/day, total dose 600 R), the reproductive ability of the males was more adversely affected than the reproductive ability of the females.¹⁵

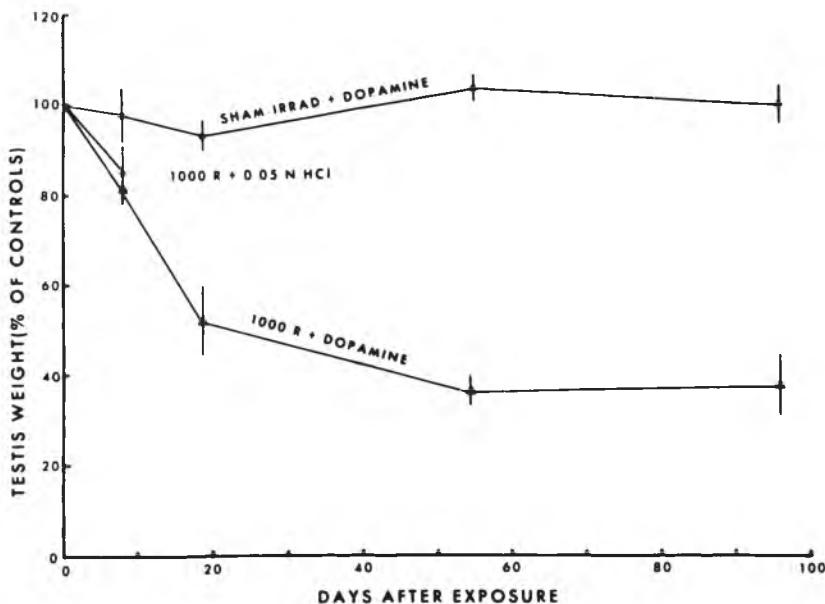


FIGURE 11.1. Changes in testis weight as a function of time after whole-body irradiation. Each rat was injected i.p. with dopamine (60 mg/kg) immediately before exposure. The normal control testes weights had an average value of 1.45 ± 0.04 , 1.72 ± 0.08 , 1.59 ± 0.14 , and $1.76 \pm 0.16 \pm$ g at 6, 18, 54, and 94 days, respectively. Each point represents the mean of at least five animals. (From Prasad, K.N. and Van Woert, M.H., *Radiat. Res.*, 37, 305, 1969. With permission.)

Rodents, cats, rabbits, and guinea pigs require a much higher dose for permanent sterility than dogs, monkeys, and humans.

Chromosomal aberration has been noted in mice whose male offspring suffered radiation damage to cells that were in premeiotic stages at the time of irradiation.¹⁶ Thus, the irradiated animals may regain complete fertility, but the offspring of such individuals may carry a significant number of chromosomal abberations.

It is difficult to evaluate the functional condition of the testes based on histological appearances; however, some correlations have been made. The testes were X-irradiated with 300 R at the rate of 50 R/day during the last 6 days of embryonic development in mice. If 40% or more tubules had all the spermatogenic elements, no sterility occurred, whereas if there were 10% or less of such tubules, sterility occurred.

Males are more radioresistant than females, on the criterion of sterility. When suckling mice were injected with 600 μ Ci of ^{131}I , so that the radioisotope reached young mice through the milk, all females were sterile, whereas only slight effects were noted in some males. In addition, an accumulative dose (440 R of X-rays at a dose of 10 R/week) produced sterility in all females, but all males were fertile.

Pretreatment of rats with 5-hydroxy-L-tryptophan (5-HTP) and 2-aminoethylisothiouronium bromide hydrobromide (AET) inhibited radiation-induced decreases in sperm counts.²⁵ Exposure to 4 Gy (400 rads) and 5 Gy γ -radiation caused a marked increase of sperm abnormality, which was significantly reduced by pretreatment with 5-HTP and AET. Radiation-induced transient sterility was prevented by the above radioprotective agents.

5. Effect on Biochemical Changes in the Testis

Very little is known about the effect of ionizing radiation on the biochemical level. This problem has been adequately discussed elsewhere.¹⁷ Testicular tissue does not form hyaluronidase after irradiation. The rate of esterification of inorganic phosphate by testicular extracts in the presence of purine nucleosides is diminished after total-body irradiation. A rise in endogenous respiration of testicular tissue occurs after X-irradiation. Treatment of X-irradiated animals with estradiol benzoate partly prevented the X-ray-induced rise in respiration, whereas treatment with testosterone propionate completely prevented the rise in respiration. Treatment of intact animals with estradiol alone produced a rise in endogenous respiration, which can be prevented by the concomitant administration of testosterone propionate.

A high percentage of abnormal sperm was observed after irradiation of bull testicles with 100–800 R.¹⁸ The changes were more marked 14 weeks after exposure. The rate of fructose utilization decreases, which correlates well with the decrease in motility. The ascorbic acid and free amino acid levels did not change.

6. Effect on Spermatozoan Metabolism

A paradoxical effect of irradiation on frog spermatozoa was observed. When ova were inseminated with sperm irradiated with lower doses of radiation, abnormal young were produced. The frequency and severity of the incidence of abnormal young increase with the increase of radiation dose. However, after a certain dose, the offspring were apparently normal. This well-known "Hertwig effect" was explained on the basis that at higher dose levels the irradiated spermatozoa merely activated the ovum for gynogenetic development, but without syngamy.¹⁹ In *Rana pipiens*, following fertilization by sperm irradiated with X-ray exposures varying from 15 to 10,000 R, only 1.6% of the embryos were viable. However, 90.5% of the embryos hatched when ova were inseminated with spermatozoa irradiated with 50,000 R. In mammals, the Hertwig effect can be demonstrated after irradiating spermatozoa with 30,000 and 100,000 R, but the development of gynogenetic embryos is highly abortive, and death soon occurs.³

The abnormalities in embryos produced from irradiated spermatozoa are generally attributed to the induction of dominant lethal mutation in the spermatozoa.

7. Effect on Sperm Motility

In rabbit spermatozoa, no change in motility was observed after irradiation with 90–6500 R; motility became very slow after 32,200 R.¹⁹ This indicates that the lower rate of fertilization from sperm irradiated with higher doses of radiation was mainly due to their reduced motility rather than to their loss of capacity to fertilize the ova.

8. Effect on Oxygen Uptake by Spermatozoa

The oxygen uptake by irradiated spermatozoa did not change, even after 100,000 R; therefore, sperm motility is more sensitive to ionizing radiation than respiration or survival. Even at 640,000 R, the average death rate in irradiated bull semen was only 31%.

9. Effect on Accessory Sex Organs in Male Animals

The accessory sex organs of the male are radioresistant. The hormone-producing interstitial cells in adult rats retain their secretory function during a 25-week period, after high exposures of 5000–10,000 R.²⁰ However, a reduction occurred in the seminal vesicle weight of adult rats that were exposed to 150 and 300 R of X-rays on the 18th day of fetal life. This indicates that the interstitial cells in the early stages of their development are sensitive to irradiation. In mice and rats, the epididymis was not damaged after 350 R of X-ray exposure.

III. FEMALE REPRODUCTIVE TRACT

Radiation not only destroys the radiosensitive gametogenic epithelium, but it also reduces the production of sex hormones. Consequently, radiation-induced sterilization may cause the production of an artificial menopause, with marked secondary effects on the more radioresistant secondary genitalia and sexual characteristics.

A. EFFECT ON THE HUMAN OVARY

The ovary ceases to contain oogonia some time after birth and contains only primary or secondary oocytes in various stages of development, which are moderately radiosensitive. However, the granulosa cells in the ovarian follicles during the rapid proliferative stage are highly radiosensitive. In growing follicles, the damage to granulosa cells is seen before changes in the oocyte.¹ Pyknosis and other degenerative changes in granulosa cells become apparent within an hour or two after moderate doses of radiation. Recovery depends upon the doses of radiation. The lower dose may allow recovery, whereas the higher dose may not.

1. Effect on Sterility

Table 11.1 shows the dose necessary to produce sterility in the female. The rate of recovery from sterility depends upon the total dose. Women whose ovaries were irradiated with small doses, at most causing only brief amenorrhea or sterility, may show complete recovery of the ovaries after 6 months. An exposure of 170 R may cause reduction or absence of mature or developing follicles after 6 months, associated with temporary amenorrhea and sterility. Such lower doses may cause premature involution over a long period after exposure. The doses, which cause permanent sterility, produce extensive damage to ovaries, including vascular sclerosis and fibrosis.

Age is a very important factor in producing radiation-induced sterility.²¹ Younger individuals (less than 40 years) require larger doses than those over 40 years in order to induce

menopause. Doses greater than 150 R may sterilize 90% of the older group, whereas only 50% of the younger group were affected until a dose greater than 500 R was delivered. Exposure of the human ovary to doses in excess of 200 R is followed by cessation of the menstrual function, which is temporary in young women but permanent in those over 40.¹ Women under 35 years who received fractionated exposures of 2000 R or more had some oocytes left in their ovaries, since pregnancy has often been reported. The exposure of the ovary with 550–650 R/day for 3 days substantially reduces urinary estrogen excretion in women with normal menstruation.²² Irradiation of the ovary in postmenopausal women also reduces urinary estrogen excretion.

B. EFFECT ON ACCESSORY ORGANS

1. The Uterus

The uterus is radioresistant. The dose to the cervix with a standard intrauterine and intravaginal radium applicator is about 10,000–20,000 rads delivered over several days. The incidence of uterine necrosis is very infrequent. Scarring and atrophy of the uterus may appear as a late effect.

2. The Vagina

The response of the vaginal mucosa to ionizing radiation is similar to that of mucous membranes elsewhere in the body.

C. EFFECT ON GYNECOLOGIC CANCER

An increase (6.6%) in the incidence of gynecologic cancer follows a low dose of pelvic irradiation.²³

D. RADIOSENSITIVITY OF THE ANIMAL OVARY

The LD₅₀ for stage I oocytes is about 8.4 R; for stage II, it is about 9.4 R. Reduction in the dose rate significantly decreased cell death in both young and adult mice.⁷ At a lower dose rate, the survival of oocytes in adult mice is much greater than that in young mice (Table 11.4).⁷ This indicates that either oocytes in young mice are more sensitive to radiation or that repair processes are more efficient in adult females. The dose-responses of oocytes are similar to those of spermatozoa.

The primordial germ cells are highly sensitive to X-irradiation. An exposure of 100 R to embryonic rats (10 days postconception) reduces the population of cells by two-thirds (70%) within 5 days of treatment. However, those cells that survive irradiation are capable of proliferation, because by 8.5 days the population has increased to about 50% of that in

TABLE 11.4
Effect of Exposure Rate on the Survival of
Oocytes in Young and Adult Mice

Age of Animals	Total Exposure (R)	Exposure Rate (R/min)	Survival (%)
Young	25	2.85	3
Young	26	0.009	13
Adult	87	0.009	28
Adult	50	87	0.13

From Prasad, K.N., *Human Radiation Biology*, Harper & Row, New York, 1974, 314. With permission.

TABLE 11.5
Radiosensitivity of Oocytes of
Mice Exposed to 20 R at Various
Times after Birth

Time of Irradiation	Decrease in oocytes (%)
Newly born	50
Day 7	86
Day 21	94

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 Harper & Row, New York, 1974, 314. With
 permission.

controls. This regenerative capacity is restricted to the primordial germ cells, because irradiation of oogonia on day 15.5 is not followed by a restoration of the cellular population.²⁴

The radiosensitivity of oocytes varies markedly with (1) age, (2) species, (3) inbred or outbred strain, (4) stage of growth of the follicle that surrounds the oocyte, and (5) chromosomal configuration. Those in primordial follicles are highly sensitive in mice, rats, and rabbits; moderately sensitive in dogs, cows, and pigs; and highly resistant in the guinea pig, monkey, and human. The response of oocytes in multilayered follicles is less variable. No histological changes occurred in the germ cells of irradiated fetal monkeys aged 4–5 months postconception following exposure of 200–400 R; however, the germ cell population was reduced when higher doses of irradiation (350–1000 R) were delivered from the second month postconception to full term (168 days) — but none of the animals had become completely sterilized.²⁴

1. Effect on Oocytes in Juvenile Mammals

The radiosensitivity of oocytes in juvenile mice increases sharply within the first few days of life (Table 11.5).²⁴ The reproductive capacity of juvenile mice is also influenced by age, and closely parallels changes in the total population of germ cells. Thus, a dose of 30 R to CF₁ mice on the 16th day induced sterility in 25% of the animals, while exposure to the same dose on day 7 or 21 sterilized only 10% of the population.

2. Effect on Oocytes in Adult Mammals

It has been shown that 90% of the total population of oocytes in mature mammals are enclosed in primordial follicles consisting of a single layer of flattened or cuboidal granulosa cells. These oocytes constitute a uniform cell population and, therefore, are important in radiobiological studies. The mortality of oocytes differs as a function of dose and also from one species to another. Amenorrhea in irradiated monkeys ensues when the population of oocytes in each ovary is reduced to 50% of the control level.

3. Factors Affecting the Differential Radiosensitivity of Oocytes

The differential response of oocytes of various species may be due to a difference in the configuration of chromosomes. Primordial oocytes of the monkey, human, cow, and rabbit contain lampbrush chromosomal loops, which are condensed and surrounded by a dense sheath of ribonucleoprotein; therefore, they are radioresistant. Conversely, the primordial oocytes of rats and mice pose highly extended (dictyate) chromosomes surrounded by a thin sheath of ribonucleoprotein, and therefore, they are radiosensitive.

The above hypothesis is supported by the following observations: (1) fluctuation in radiosensitivity that occurs during the juvenile period in rats and mice may be related to

changes in chromosomal configuration, and (2) the sharp change from the highly sensitive small oocytes to resistant stage II cells in rats is accounted for by a reversal in chromosomal configuration from dictyate to diplotene.²⁴

IV. SUMMARY AND COMMENTS

The gonads are generally extremely radiosensitive. Although considerable data on the effect of irradiation on the reproductive tract are available, data on human gonads — especially on the testis — are limited because the testes are rarely irradiated in clinical settings. In the testis, spermatogonia are most sensitive to radiation on the criterion of cell death, whereas spermatids and spermatozoa are highly radioresistant. In the male, radiation-induced sterility is never immediate because of the high radioresistance of spermatids and spermatozoa. The male accessory organs are highly radioresistant. Animal studies have shown that, for sterility, the dose rate is important. The lower the dose rate (within a certain dose rate range) the higher the sterility in the male. Short-term fractionated doses produce a higher incidence of sterility in males than does a single dose. Pretreatment with radioprotective agents can reduce radiation-induced changes in the testis.

In the female, in contrast to the male, radiation not only destroys the radiosensitive gametogenic epithelium, but also much of the production of sex hormones. In general, in growing follicles, postirradiation damage to granulosa cells is seen before changes in oocytes. The female is more sensitive than the male on the criterion of sterility. Unlike the male, sterility in the female is immediate. Age is a very important factor in producing radiation-induced sterility. The younger individuals (less than 40 years) require larger doses than those over 40 years to induce menopause. The uterus is highly radioresistant. The response of the vaginal mucosa is similar to that of mucous membranes elsewhere in the body. The phases of acute erythema, moist desquamation, and mucositis are clinically similar to those in oral mucosa. The tissues of the vulva, labia, and clitoris are relatively more radiosensitive. The normal mammary glands are radioresistant.

Animal studies show that a reduction in dose rate significantly decreases cell death in both young and adult mice. At a lower dose rate, the survival of oocytes in adult mice is much greater than that in young mice. The sensitivity of ovarian stem cells in the rat increases to a maximum when the incidence of mitosis in oogonia is highest. The sensitivity of oocytes to radiation varies as a function of the stage of development. In addition, the radiation response of oocytes varies considerably with (1) age, (2) species, (3) inbred and outbred strains, (4) stage of growth of the follicles that surround oocytes, and (5) chromosomal configuration. It has been suggested that the variation in radiation response of the oocyte is due to a variation in chromosomal configuration. If the oocytes have lampbrush chromosomal loops that are condensed and surrounded by a dense sheath of ribonucleoprotein, they are radioresistant; however, if the oocytes have highly extended chromosomes surrounded by a thin sheath of ribonucleoprotein, they are radiosensitive.

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Chapter 12

RADIATION DAMAGE OF OTHER ORGAN SYSTEMS

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I. THE CARDIOVASCULAR SYSTEM

A. EFFECT ON HEART

The radiation response of the heart is called radiation carditis. The myocardial cells are highly radioresistant on the criterion of morphological changes. The structural changes in the myocardium are seen only after high-radiation therapeutic doses. The pericardial cells are relatively more radiosensitive; the damage to the pericardium is produced with more frequency at lower radiation doses. The fine vasculatures are relatively radiosensitive; therefore, acute and chronic effects of irradiation of the heart are primarily due to the damage of the vascular system.

Radiation-induced alterations include swelling of the fiber, loss of longitudinal and cross-striation, homogenization of the sarcoplasm, complete disappearance of protoplasm, and persistence of hollow sarcolemma.¹ Nuclei may show pyknosis, fragmentation, and lysis. The intima of small arterioles may become thick.

Myocardial infarction rarely occurs during or after radiation therapy. There have been frequent reports of pericarditis, pericardial effusions, pericardial adhesion, and pericardial fibrosis. The degree of damage and extent of recovery depend upon radiation doses and other physical and biological factors.

In animals, also, high doses of radiation are required to produce damage in the heart. In the dog heart, myocardial necrosis and hyaline degeneration have been observed after 38,000 R was given to the heart over 8 weeks.³ The tolerance dose for rat myocardium is about 5000 R. Myocardial hemorrhage, together with epicardial and endocardial hemorrhages, was reported in irradiated monkeys that had received 700 or 800 R of whole-body irradiation.⁴

B. EFFECT ON AORTA AND MAJOR VESSELS

Hemorrhage is the main manifestation of damage to the major vessels during or shortly after the completion of radiation therapy. Doses in the range of 5000 R in 41–70 days have produced rupture of the aorta.¹ The damage of blood vessels may cause thickening of intima and formation of thrombi.

C. EFFECT ON MEDIUM- AND SMALL-SIZED VESSELS

The medium-sized vascular arteries also show degenerative changes similar to those seen in large vessels, but they are not as severely damaged as are arterioles and capillaries.¹ The repair of acute injury to the blood vessel walls by fibrosis may cause an advanced degree of vascular sclerosis. Excessive proliferation of cellular elements may completely block the lumen of the vessels.¹

Animal experimentation has shown that the small blood vessels and capillaries are more radiosensitive than the large vessels. In general, the endothelium is the most sensitive part of the blood vessels, and the vascular changes are more marked within the organs than in the arterial trunk. Radiation-induced damages are not identical in all the vascular segments; they may vary from one artery to another in the same animal.¹³

II. THE RESPIRATORY SYSTEM

The radiation response of the respiratory system is called radiation pneumonitis. The richly ramified vascular system and lymphatic tissues are radiosensitive, but cartilage of air passages and the pleura are radioresistant. It has been shown¹ that pulmonary infection renders lung tissue more radiosensitive. In humans, radiation pneumonitis has been observed 4–6 months after a fractionated exposure of 3000–5000 R. Radiation pneumonitis may be regarded as an

inflammatory reaction which may recover or may lead to chronic lung fibrosis. It is characterized by the accumulation of fibrin-rich exudate within alveoli, the thickening of alveolar septa by fibrillar material, and cellular proliferation. The fibrin condenses at the alveolar walls to produce the so-called "hyaline membrane." Fibrin membranes were present in 41% of all irradiated lungs 6 months to 2 years after exposures greater than 2000 R.⁵ It was further observed that increased deposition of fibrillar connective tissue in the alveolar septa was most frequent after radiation exposures greater than 3000 R; the latent period was longer than 6 months. The increased accumulation of histiocytes and fibroblasts in the alveoli was seen most frequently at exposure levels between 2000 and 5000 R. In some cases, fibrosis of the alveolar walls (with dense fibrous tissue) could be found a month or two after relatively large doses (5000 R), but most frequently it is present after 6 months or more. The clinical signs of lung fibrosis may appear several years after irradiation.

The volume factor is very important in the radiation response of the lung. If less than 25% of the lung is irradiated with a fractionated total dose of 3000 R, no clinical effects are seen. If larger areas of the lung are irradiated, then radiation pneumonitis is more severe. If both lungs are irradiated at exposures of 3000 R and above, it is generally fatal.

The dose rate also affects the severity of radiation pneumonitis. At a dose rate of 1000 rads/week, acute reactions are fairly common; when the dose rate is reduced to 700 rads/week, this reaction is unlikely. For lung fibrosis, the total dose is very important; however, the dose rate has no effect in determining radiation response of the lung. The presence of chronic pulmonary disease, such as interstitial fibrosis, pulmonary emphysema, and pneumoconiosis, probably increases the radiosensitivity of the lung.

Age may also be an important factor in the radiation response of the lung. A child who received irradiation of both lungs with a midthoracic dose of 1700 R for metastatic Wilm's tumor died of radiation pneumonitis. Hyperlucent lung, in a survivor of Wilm's tumor, has been observed after a fractionated dose of 1500 R.⁶

III. THE URINARY TRACT

A. EFFECT ON HUMAN KIDNEY

The kidney is a moderately radiosensitive organ. The radiation response of the kidney is called *radiation nephritis*, which occurs 6 months to a year after the completion of radiation therapy. The major complaints are swelling of the legs, shortness of breath, headache, and vomiting.

1. Effect on Vascular Changes

The main features of radiation nephritis are an early hyperemia and increased capillary permeability, which result in interstitial edema. Endothelial cells of the fine vasculature show degeneration and necrotic changes, followed by a rapid proliferation, which eventually may block the lumen of the vessels. The kidney cortex becomes pale, probably due to the blockage of the afferent glomerular arterioles.

2. Rapid vs. Slow Vascular Occlusion

Rapid mechanisms of occlusion of the small vessels include endothelial swelling and proliferation, and thrombosis. Slower mechanisms involve the progressive degeneration and thickening of vessel walls and the narrowing of lumens. The cortical ischemia, produced by a slow or rapid mechanism of occlusion, may cause degeneration of the tubular epithelium, which is gradually replaced by connective tissue. The convoluted tubules appear to be more sensitive to ischemia than the medullary tubules.

The occurrence of hypertension depends upon radiation doses. With larger doses, the hypertension may develop earlier and to a greater degree. Hypertension causes degeneration and thickening of the small arterioles and the larger vessels of the kidney. Previously irradiated renal vessels are more susceptible to hypertension-induced necrosis than are renal vessels not previously irradiated.¹ Three young children died of renal failure and hypertension within 2 months after therapeutic doses (total doses of 5200–6850 R in about 4 months) over the renal area.⁷ Five adults died of acute radiation nephritis between 5 and 16 months after therapeutic doses; the dose to the kidney was 2300 R or more in 5 weeks or less.

3. CHRONIC RADIATION NEPHRITIS

Chronic radiation nephritis may develop slowly, with a mean latent period of about 2–3 years after exposure. The main pathological changes include progressive nephrosclerosis, degeneration of fine vasculature, sclerosis of arterioles and arteries, degeneration and sclerosis of glomeruli, hypertrophy of surviving glomeruli, degeneration and atrophy of tubules, and advanced interstitial fibrosis.¹ Chronic radiation nephritis may be associated with “benign hypertension.”

An exposure of 2800 R delivered to the entire area of both kidneys may cause hypertension and renal failure;⁸ irradiation of a smaller volume of kidney may reduce that risk.

Age does not appear to be an important factor in radiation nephritis. However, when unilateral nephrectomy is performed, the remaining kidney becomes more radiosensitive — probably because of a stimulus to cell division. A 9-month-old infant who received 2000 R during radiation therapy of Wilm’s tumor after unilateral nephrectomy died 15 days after exposure.⁹

B. EFFECT ON ANIMAL KIDNEY

It has been reported that renal function and renal hemodynamics were not significantly modified in dogs that were exposed to 500 R of whole-body X-irradiation.¹⁰ However, in the uninephrectomized, irradiated dog, the renal oxygen consumption and urea clearance were significantly increased, and the plasma concentrations of nonesterified fatty acid and lactate were decreased, 6 days after irradiation. It has been reported that the renal concentrating capacity in dogs became impaired 2–3 weeks after the irradiation of the kidneys (a single dose of 2500–3000 R).

In addition, the concentrating ability of the kidney fell before the glomerular filtration rate (GFR) decreased. In these animals, both the GFR and the renal plasma flow (RPF) were markedly reduced. A decrease in renal cortex alkaline phosphatase activity after irradiation (600–2400 R) may not be due to the direct effect of irradiation on the kidney, since local irradiation of the kidney with similar doses did not produce any change in the enzyme activity.¹ The kidneys of three dogs with unilateral nephrectomy were exposed to 2010, 2750, and 3780 R in 13 weeks. Functional impairment consisting of *p*-aminohippuric acid (Tm-PAH) depression, deficient insulin clearance, and reduced renal blood flow was also observed. Radiation-induced renal atrophy was characterized by scarring.

C. EFFECT ON BLADDER AND URETERS

The radiation responses of the bladder and ureters are referred to as *radiation cystitis*. The symptoms of acute cystitis are dysuria and nocturia; the bladder capacity is reduced. The continued obstruction and infection, if unabated, may cause bilateral hydronephrosis, pyelonephritis, uremia, and death.

The pathological changes in acute radiation cystitis are analogous to acute radiation dermatitis.¹ Primary erythema develops in 24 hr or less and secondary erythema appears in 3–4 weeks. The changes in the bladder are characterized by hyperemia, congestion, edema,

degeneration of epithelial cells, desquamation of the epithelium, and damage to fine vasculature and connective tissue. If the radiation doses are large, there may be varying degrees of ulceration. Extensive necrosis favors the growth of urea-splitting organisms, the formation of calcerous deposits, and sometimes calculi. Depending upon the degree of damage to vascular and connective tissues, ulcer of the bladder wall may heal slowly by epithelial regeneration and/or scarring, or it may result in *fistula formation*. The presence of infection increases the radiosensitivity of the bladder.

1. Radiosensitivity of Ureters

The ureteral epithelium is relatively less radiosensitive than the bladder epithelium. The sequence of changes after irradiation is similar to that seen in the bladder.

IV. EFFECT ON LIVER

Liver is a highly radioresistant organ on the criterion of morphological changes. The characteristic acute radiation response of the liver is referred to as *radiation hepatitis*.

A. RADIATION HEPATITIS IN THE ADULT

Doses below 3000 rads may not produce liver damage, whereas doses above 4000 rads produce damage in 75% of patients.¹² The characteristic responses of radiation hepatitis are severe sinusoidal congestion, hyperemia or hemorrhage, some atrophy of the central hepatic cells, and mild dilation of central veins.

B. RADIATION HEPATITIS IN CHILDREN

Children's livers are more radiosensitive than those of the adults on the criterion of radiation hepatitis. A dose of 2000 rads of γ -radiation divided over a 2-week period may cause radiation hepatitis. When the radiation was delivered in combination with dactynomycin for the treatment of Wilm's tumor, the children's livers developed more severe radiation hepatitis.¹³

C. RADIATION-INDUCED LIVER NECROSIS

Necrosis in the liver is observed several years after the completion of radiation therapy. This may be primarily due to damage of the fine vasculature and connective tissue. In rabbits, small necrotic patches¹⁴ may be seen 2 days after large doses (30,000 R). Among survivors of dogs that received 800–5250 R to the liver, fibrosis was observed as early as 6 weeks after exposure. One dog that died 16 months after irradiation showed foci of necrosis and portal fibrosis. In another dog, 7 months after irradiation, advanced centrolobular necrosis, fibrosis, and calcification were seen. Deep jaundice also developed in some of the irradiated dogs. Nutritional conditions may influence the radiosensitivity of the liver. It has been demonstrated¹⁵ that a dose of 450–500 R total-body irradiation acted synergistically with a 6% casein diet to cause a high incidence of severe hepatic necrosis in rats.

D. RETICULOENDOTHELIAL ELEMENTS

A total dose of 3300 R (50 R/day) caused hypertrophy of Kupffer cells¹⁶ in the rat liver. Reticular fibers of the stroma showed changes after 7400 R. The fibers of the reticulum network that entwine the hepatocytes were swollen and thickened.

E. REGENERATING LIVER

The regenerating liver is highly radiosensitive, like any proliferating system; however, the liver maintains the capacity to regenerate even after a high dose of irradiation. When the

residual lobes of the liver received 20,000 R immediately after partial hepatectomy, regeneration took place almost to the control level (90% of the control liver weight).

F. FUNCTIONAL CHANGES IN THE LIVER

Irradiation of the liver causes a decrease in the glycogen content, but an increase in the glucose content.¹⁷ The mechanism of this effect is unknown. Lipogenesis is not affected by irradiation of liver alone.

V. EFFECT ON BILIARY DUCTS

It has been suggested that, in humans, epithelial cells of the biliary ducts — particularly the small and medium ducts — appear to be more radiosensitive than the liver cells.¹⁸ The secretion of bile salt in the dog, even after a high dose, was unaffected.¹⁷

VI. EFFECT ON PANCREAS

The pancreas is relatively radioresistant. The insular epithelium is more radioresistant than the acinous epithelium. Pyknosis and necrosis of the islet beta cells were observed in monkeys,¹⁹ who died within 8 days after doses of 10,000–50,000 R of whole-body irradiation. Necrosis of the islet cells in rats was evident 8 hr after doses of 2500–5000 R. The alpha cells were more sensitive (LD_{50} about 5000 R) than the beta cells (LD_{50} about 20,000 R). The acinous cells showed no demonstrable morphological changes within the same period.

The exteriorized fragment of the dog pancreas was irradiated with 5000–9000 R. Reduction in the size and number of zymogen granules becomes prominent with time, but later these granules reappeared. Most of the ultrastructural alterations disappeared during the recovery phase; however, a mild inflammatory reaction and moderate fibrosis persisted.²⁰ There were marked decreases in the pancreatic amylase, leucine aminopeptidase, and lipase activities. A preirradiation dose of alloxan monohydrate (15 mg/kg of body weight) completely protected the dog pancreatic islet tissue ultrastructures.

VII. EFFECT ON GLANDS

A. EFFECT ON SALIVARY GLANDS

The salivary glands may be exposed to irradiation during the radiation therapy of oral tumors. They are generally radioresistant on the criterion of cell death. The most consistent and common effect of irradiation of adrenal glands is dryness of the mouth within 4–6 hr. Enlargement of the parotid and submaxillary glands usually starts at this time, and the gland rapidly reaches an enormous size. This process peaks in 12–24 hr. The oral mucosa is erythematous, and patchy mucositis may develop.¹

When fractionated doses (100–300 R/day) are delivered over the parotid or submaxillary glands, the swelling of these glands rarely occurs, and there is no effect on saliva secretion.¹ If radiation is given in therapeutic doses for 3–4 weeks, the saliva becomes more viscous and frothy because of increased mucous secretion. This results in cessation of secretion and drying of the oropharyngeal mucosa.

A single dose of 100–400 R to the human salivary glands results in an increase in the serum amylase level.²¹ The amount of increase is dependent upon the total volume of salivary gland

irradiated and the total radiation dose. Larger doses and volume irradiated produce a greater increase in serum amylase activity.

Animal experimentation using 1000–2500 R has shown²² that of all three glands, the acini are the most radiosensitive and the excretory ducts the least, whereas the secretory tubules and intercalated ducts are moderately radiosensitive. Vascular and inflammatory reactions and subsequent fibrosis commonly occur after irradiation. Fibrosis is more severe in the parotid gland than in the other glands. The irradiated blood vessels also show narrowing of the lumens.

After 2500 R, the acinar mass shrinks progressively, and the loss of cells is never fully restored by regeneration of the acini, secretory tubules, and intercalated ducts.²² Adenomas of the acinar type are observed after irradiation. The incidence of acinar adenoma is dependent upon dose, fractionation, and sex of the animal. The sublingual glands in males are particularly sensitive to radiation-induced adenomas. All animals exposed to 2550–4250 R develop adenomas after 3–5 months.

In the submandibular gland, there is an inverse relationship in males between the compensatory hypertrophy of secretory tubules and the incidence of acinar adenomas. The peak of tubular hypertrophy is reached at 3400 R, and no adenomas are observed; while with larger doses, the adenomas appear in varying incidence (14–67%).

In the parotid, no adenomas are observed if the radiation dose is delivered within a short time; however, they are found if the radiation exposure of 850 R is given at intervals of 2 months over a period of 6 months. The adenomas are of tubular rather than acinar type. The prolonged radiation exposures favor the development of adenomas in the parotid and submandibular glands, but not in the sublingual glands.

B. EFFECT ON ADRENAL GLANDS

Adrenal glands are highly radioresistant on the criterion of cell death. In humans, an exposure of less than 2000 R caused no alteration of adrenal steroidogenesis 1 or 2 days after i.v. adrenocorticotropic hormone (ACTH) administration, whereas exposures to more than 2000 R caused an increase in adrenal cortical steroidogenesis 1 day after an ACTH injection.²³ The normal amount of steroidogenesis under nonstress conditions did not change, even after a high exposure (3500 R to the adrenals), but the ability of the adrenal to respond to stress is markedly reduced. The plasma hydrocortisone concentration did not change, even after a high dose of therapeutic radiation. However, when the adrenal glands were directly exposed to 150 R, a transient and sharp fall of plasma hydrocortisone concentration occurred, but recovery was rapid.²⁴

Animal experiments have shown that the adrenal cortical activity increased during acute radiation sickness. After irradiation of the rat abdomen, or head and neck, with 2800 R, the adrenal weight increased about 30% in 48 hr.²⁵ After whole-body exposures of 650–900 R, the cortical cholesterol content was markedly reduced in 4 days and returned to the normal level in about 10 days. The mitotic index of mouse adrenal cortical cells was depressed by X-irradiation (325 R). After whole-body exposure (800 R) the synthesis of corticosteroid increased in the rat adrenal 2.5 hr after irradiation, and the secretion increased 72 hr after irradiation; both of these phases were inhibited by preirradiation treatment with a radioprotective compound (cysteamine).

C. EFFECT ON THE THYROID GLAND

The normal thyroid gland is generally exposed to irradiation during radiation therapy of malignant head and neck tumors or the treatment of hyperthyroidism with ^{131}I . The thyroid gland is radioresistant on the criterion of morphological change. The damage to the thyroid is primarily due to damage of the vascular system.¹ High doses of radiation may produce

edema, inflammation, and hemorrhage in the thyroid, probably due to damage to the vasculature. The colloid contents of damaged follicles become reduced.¹ The follicular basement membrane may rupture. After moderate doses, the regeneration of the follicular epithelium may be rapid.

Patients who received doses (1000–4000 rads) to the thyroid for the treatment of other malignant diseases developed hypothyroidism a few months to many years after completion of radiation therapy.¹ The moderate doses of radiation (1000–2000 rads) may reduce the function of the thyrotoxic gland to the level of euthyroidism. It has been estimated²⁷ that a dose of 50,000 rads (1 mCi of ^{131}I per gram of thyroid tissue would give a thyroid dose of 50,000 rads) is required to destroy the thyroid completely.

The radiosensitivity of the thyroid is influenced by its metabolic activity. The more active glands, therefore, are more radiosensitive. Children's thyroids are more radiosensitive than those of adults.

Animal experiments have shown²⁸ that the delayed radiation-induced primary hypothyroidism in adult dogs develops in about 2–4 years after radiation exposures of 1000–2100 R. The lower exposures increased the latent period of hypothyroidism. The primary changes involved damage to the fine vasculature and connective tissue. Fast-neutron irradiation of the rat thyroid with 300 rads inhibited its function after 24 hr.²⁵ Pregnant mice injected in late gestation with 200 μCi of ^{131}I resulted in complete thyroid destruction 1–11 weeks later. Adult rats receiving up to 30 mCi of ^{131}I showed only a few damaged follicles, increased epithelial mitoses, and compensatory thyroid hypertrophy. After large doses of 60–78 mCi (equivalent to 20,000 R), the thyroid function decreased.²⁵

D. EFFECT ON PARATHYROID GLANDS

The parathyroid glands are also highly radioresistant on the criterion of morphological changes. Very little information on the effect of irradiation on the parathyroid gland is available. Partial parathyroid insufficiency, after treatment with ^{131}I for hyperthyroidism, was found in 7 of 12 patients given a hypocalcemic challenge test. The serum calcium level returned to the normal level quite slowly.²⁹

VIII. EFFECT ON MUSCLES AND BONES

A. EFFECT ON MUSCLE

Human striated muscle is highly radioresistant on the criterion of morphological changes; however, delayed necrosis of muscles has been observed³⁰ after therapeutic doses. The delayed necrosis of muscle may be primarily due to damage of blood capillaries and connective tissue. A cumulative dose of 4500 R produced detectable damage in the muscle after 3 years in patients with good general health. However, in patients suffering from carcinomatous cachexia or other debilitating diseases, muscle necrosis was constantly observed following exposure of 2000–5000 R after a similar latent period. Children who survive radiation therapy for a malignant disease may show muscular atrophy after a long latent period. This is seen in the back muscles of long-term survivors of Wilm's tumor and in the neck muscles of survivors of Hodgkin's disease, after radiation therapy.

In rats exposed to a single dose of radiation, the muscle lesion produced by 5150 R of X-rays was similar to that produced by 3500 rads of 47.5-MeV protons, the RBE value for proton being about 1.5. It has been suggested that the delayed necrosis of muscles precedes the development of delayed necrosis of the spinal cord.

A thyrotoxicosis condition produces muscle necrosis within 5 months after 2200 rads, whereas the control, irradiated with the same dose, did not show any muscle lesion over a

period of 2 years. A subthreshold ultrasonic radiation dose accelerated the appearance of delayed radionecrosis of the spinal cord, but had no such effects on the axial muscles.

B. EFFECT ON CARTILAGE AND BONE

1. Growing Cartilage and Bone

Growing bones and cartilage are relatively more radiosensitive. Several studies have shown that the growth of bone and cartilage of young children is impaired after therapeutic doses of radiation.¹ Children exposed to 50–500 R for benign conditions of the neck, such as adenitis and enlarged thymus, show detectable radiographic changes in bone and cartilage in comparison to a control group. The cervical spine was altered, and osteophytic formation and diminution of disc interspaces were observed. Mandibular growth impairment was also seen in some cases, causing facial asymmetry.¹

In the growing long bones of the rat, an exposure of 2400 R causes a complete cessation of chondrogenesis and osteogenesis. The bone growth stops within a few days. The shaft becomes extremely delicate in appearance, fractures readily, and resembles severe osteogenesis imperfecta. The severity of effect varies directly with dose. The fractionation or protraction of dose reduces the severity of the effect. The degree of bone damage also depends upon the extent of damage to the fine vasculature and their relative rates of recovery. The minimal stunting dose (MSD) varies as a function of the age of the organism. The MSD value for the long bones of rats 30 days of age is about 600 R.³¹ The MSD in older bones produces more damage because of less recovery than the MSD in younger bones.

The early changes in growing cartilage following irradiation include (1) reduction in mitotic index of chondroblasts, (2) degeneration or necrosis of chondroblasts, (3) reduction in the number of chondroblasts, and (4) enlargement of lacunae. An exposure of 600–1200 R causes submaximal damage to the cartilage plate. Such exposures permit sufficient recovery of the chondroblasts, vasculature, and bone growth; however, some degree of residual damage, depending upon the dose, may persist. Larger doses (1200–3000 R) cause maximal damage to chondroblasts, fine vasculature, and cellular elements of bone marrow. Recovery of chondroblasts and fine vasculature may be slow; therefore, the cartilagenous matrix of the cartilage plate becomes dense and more brittle.¹

2. Osteogenesis vs. Chondrogenesis

The osteoblasts are much more resistant to radiation damage than the proliferating chondroblasts, and consequently osteoid deposition and osteogenesis may continue after cartilage growth has stopped.

3. Mature Cartilage and Bone

Mature cartilage and bone are very radioresistant. Necrosis of bone and cartilage may occur long after irradiation with high therapeutic doses (greater than 5000 rads). The necrosis results primarily because of damage to the fine vasculature. Old people may be subject to necrosis of the bone at a lower dose level than young individuals, because the vascular system in the elderly is already impaired.

One of the most important delayed effects of radiation damage to bone is susceptibility to fracture from strain or traumatic injury.¹

IX. EFFECT ON TEETH

Hypersensitivity of the teeth to heat, cold, and sweet food may be observed 6–8 months after the completion of radiation therapy of oral cancer.¹ Decay of the neck of the tooth may

also be noted. Some studies have shown that a marked retardation in tooth and bone development occurs several years after therapeutic irradiation to oral areas in children during the first few years of postnatal life.¹ In rats, an exposure of 25 R to the growing teeth causes detectable damage.

X. EFFECT ON EYES

The eyes consist of complex structures that show variation in radiosensitivity. The lens is radiosensitive, the conjunctiva and cornea are moderately radiosensitive, and the retina and optic nerve are radioresistant. After therapeutic radiation doses (6000–7000 R) to the orbit, conjunctivitis appears and photophobia becomes pronounced. The secretion of the lacrimal gland is suppressed, and the eye is bathed in thicker secretions and is more readily irritated and subject to secondary infections. The normal mucous membrane of the conjunctiva is converted into a roughened keratinized surface.¹ The retina of the young is more radiosensitive than the retina of older individuals (LD_{50} for young retina cells is about a few hundred roentgens, and for old retinal cells it is about a few thousand roentgens). However, in the mature retina, necrosis may develop secondary to the damage of the fine vasculature. The rod cells seem to be more susceptible to such damage than the cone cells, whereas bipolar and ganglionic cells are radioresistant. Massive doses of radiation can cause complete blindness.

The lens is a highly radiosensitive structure and shows changes after exposure. Inhibition of mitosis and death of epithelial cells temporarily interrupt and interfere with the progression of differentiation and deposition of lens fibers. The degree and duration of this process of lens fiber disorganization determine the degree of opacity or cataract. The RBE of α particles and deuterons for the inhibition of mitosis is 4.1 and 3.9, respectively.

XI. EFFECT ON EAR

The early effect of radiation on the middle and inner ear consists of damage primarily to the capillaries and fine vasculature. The early radiation responses are referred to as an acute "vasculitis of the middle ear" (otitis media) and inner ear (labyrinthitis). These changes may cause disturbances in the cochlear function, which leads to an abnormal sensitivity to loudness.¹ Infection may complicate the effects of irradiation on the middle ear. Uncomplicated radiation otitis media usually subsides a few weeks after irradiation. The progressive degenerative changes in blood vessels and in connective tissue may seriously impair the blood supply of the cochlea, labyrinth, and auditory ossicles, which may result in degeneration and fibrosis of dependent structures and loss of hearing capacity. The delayed necrosis of the auditory ossicles has been described as a late effect.

The LD_{50} for acute radiation otitis is between 4000 and 6000 R, which is the range commonly reached in radiation therapy of head and neck cancers.³² In a 2-year-old child, serious otitis media developed after a therapeutic dose of 2000 R.¹ A study with animals indicates that a local exposure of 7100 R in 30 days produced radionecrosis in the internal ear in 50% of rabbits.³³ The tolerance doses in the rabbit have been suggested to be 1400 R in a single dose, 3000 R in 12 days, and 4500 R in 30 days.

XII. SUMMARY AND COMMENTS

Most of the organ systems described in this chapter are radioresistant on the criterion of morphological changes; however, their fine vasculature and connective tissue are relatively

radiosensitive. The delayed necrosis observed in various organs long after the completion of radiation therapy is primarily due to damage to the fine vasculature and connective tissue. The early reactions are primarily of an inflammatory nature. The extent of damage and recovery depends upon the degree of damage to the vascular system and connective tissue, which, in general, is dependent upon the total dose. The precise dose-effect relationship for all the effects mentioned in this chapter has not been established in humans; however, data from clinical experiences and animal experiments provide some estimation of the dose-effect relationship.

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Chapter 13

RADIATION IMMUNOLOGY

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I. INTRODUCTION

Ionizing radiation suppresses the immune response of the whole organism. It does so either by inhibiting innate immunity, which includes destruction of mechanical barriers, cellular defense mechanisms, clearance mechanisms, bactericidal efficiency of the blood, and the properdin system, or by inhibiting the formation of antibodies.¹ The humoral and cellular responses after irradiation have been extensively reviewed.²

II. EFFECT ON INNATE IMMUNITY

A. MECHANICAL BARRIERS

It has been shown that high doses of irradiation can impair the barrier function of certain tissues that normally prevent the penetration of an infective agent into the bloodstream. Examples of such tissues are the skin, lung, and intestinal mucosa.⁷

B. CELLULAR DEFENSE MECHANISMS

Radiation damages the phagocytic mechanisms. For example, intestinal microorganisms are found in spleen and liver cultures of mice 60 hr after whole-body irradiation (1200 R). This shows that phagocytic cells in the spleen and liver were unable to engulf and destroy the microorganisms. The macrophages are markedly resistant.¹ Therefore, the suppression of their phagocytic activity after irradiation may be due to a blockade caused by the ingestion of large quantities of cellular debris or due to an alteration in the membrane.

C. CLEARANCE MECHANISM

The natural resistance of organisms to certain infections depends on their ability to eliminate the infective agent from their bodies within a short time. Ionizing radiation impairs this ability. For example, rabbits are resistant to dysentery bacillus. When they are exposed to a whole-body exposure of 800 R, prior to the administration of the microorganisms, most of them die. However, the survivors carry these microorganisms a long time after exposure. This indicates that the irradiated rabbits are unable to completely eliminate the microorganisms from their bodies.

D. BACTERICIDAL EFFICIENCY OF THE BLOOD

It has also been found that ionizing radiation impairs the normal bactericidal activity of rabbit serum.¹

E. THE PROPERDIN SYSTEM

In some animals, the production of properdin, a serum protein, takes place without any antigenic stimulation.¹ This serum protein participates in the destruction of bacteria and in the neutralization of viruses. The properdin level, in dogs lethally irradiated with high doses, decreases during the postirradiation period. However, the properdin level increases in survivors. Zymosan, which alters the properdin level *in vivo*, provokes a double increase in the leukocytes of irradiated dogs at the time of maximum leukopenia.¹ It has been reported that properdin partially protects the rats irradiated with the $^{30}\text{LD}_{90}$ dose.

III. MODIFICATION OF THE EFFECT OF IRRADIATION OF INNATE IMMUNITY

The administration of antibiotics to animals irradiated with a bone-marrow syndrome dose reduces the inhibitory effect of radiation on innate immunity. The antibiotic does not directly stimulate defense mechanisms, but it helps in the recovery of the irradiated animals.

IV. EFFECT OF IRRADIATION ON ANTIBODY FORMATION

Actively acquired immunity is another defense mechanism. Synthesis of specific antibodies is induced by the penetration of antigens. Well-known antigens include proteins, polysaccharides, and whole cells such as bacteria and foreign cells. Antibodies agglutinate bacteria

or dissolve them. The effect of radiation on antibody production depends on whether the organisms have previously been in contact with antigens (secondary response) or whether they are in contact for the first time (primary response).

A. EFFECT ON PRIMARY RESPONSE

Primary response is generally more radiosensitive than the secondary response. Ionizing radiation reduces the formation of antibody. The extent of decrease is associated with the dose, time of antigenic stimulus, and nature of the antigen. It has been reported³ that depression of the primary hemolysis response occurred in rats when 300 R was given at various times during the week, before and 5 days after a single injection of sheep erythrocytes. An X-ray exposure of 175 R given 3 days before the antigen caused a greater depression of the hemolysin response than 600 R given 2 days after the antigenic stimulus.^{3,4} The relationship between the effect of irradiation on hemolysin formation and the time of antigen is summarized below:⁵

1. The maximum reduction of antibody formation occurs when irradiation was given 12–24 hr before the administration of the antigen.
2. When the antigen is administered just after irradiation, the amount of antibodies produced is almost equal to normal values, but the production rate is less.
3. On administering the antigen shortly before irradiation, the results are similar, but the antibody production rate is higher than normal.

Similar results were obtained by other authors using other antigens and other species of animals. Certain quantitative differences appear to be due to the differences in species, in the half-life of antigens, and in the molecular weights of antibodies that may have different rates of metabolism in the body.

It has been suggested⁶ that the formation of antibodies involved three phases: (1) the preinduction period, (2) the induction period, and (3) the production period. The radiosensitivity of these periods differ.

1. *Preinduction period:* This period covers a brief event that is essential for initiating the development of the antibody-synthesizing mechanism. This phase is highly radiosensitive and appears to last for 1–4 hr. The dose-response curve for this criterion of damage is a typical sigmoid type.
2. *Induction period:* This period is relatively less radiosensitive. Irradiation does not affect the total quantities of antibodies produced, but their rates are slower. In some cases, the maximum titers are actually higher than in the nonirradiated controls.
3. *Production period:* The production period is highly radioresistant. The antibodies produced by irradiated rabbits do not differ immunochemically from those of normal rabbits.⁸

B. EFFECT ON SECONDARY RESPONSE

The secondary response is relatively more resistant to irradiation than the primary response. A dose of 153 rads causes a 50% reduction in the primary response, whereas a dose of 437 rads is needed for a similar decrease in the secondary response.⁵ Some have regarded the secondary response and primary response as equally radiosensitive.

V. RADIOSENSITIVITY OF VARIOUS TYPES OF LYMPHOCYTES

Generally, lymphocytes are considered to be one of the most radiosensitive cells in the human body; this may account for the radiosensitivity of immune response. Among the various types of lymphocytes, B cells and unprimed T cells are very radiosensitive, whereas

macrophages, carrier-primed T cells, and plasma cells are radioresistant. The lymphocytes in the thymus, spleen, and lymph nodes are also very sensitive to whole-body irradiation.

B lymphocytes in mice are relatively more radiosensitive than T lymphocytes after acute or chronic exposure.⁹ The rate of recovery was similar after chronic exposure.⁹ It has been reported⁹ that chronic irradiation caused an immunosuppression that persisted throughout the adult life of the animals. The number of splenic T and B cells in these mice returned to normal. The phenomenon of cap formation, a process involving surface membrane components, was adversely affected in the surviving B cell population, but not in the surviving T cell population.⁹ This defect also recovered. Thus, the persistent immunodeficiency in chronically irradiated mice may be due to the fact that the microenvironment of the irradiated spleen alters factors regulating T and B cell interaction in response to a T-dependent antigen.

A study¹⁰ has proposed that the spleen of mice that have been thymectomized, lethally irradiated, bone-marrow reconstituted, and which were primed with xenogeneic erythrocyte antigens prior to irradiation and bone marrow injection, contains three types of functionally unique T cells: (1) radiosensitive classical T cells, (2) radioresistant helper T cells, and (3) radioresistant residual "virgin" T cells that have the potential to proliferate when stimulated with an appropriate stimulus. Therefore, it has been suggested¹⁰ that putative "virgin" T cells surviving in the spleen of lethally irradiated bone-marrow-reconstituted mice could initiate an immune response.

Natural killer (NK) cells are naturally occurring cells that destroy a variety of tumor cells and cultured cell lines. Therefore, they are considered to play an important role in immunosurveillance mechanisms against spontaneously occurring tumor cells *in vivo*. It has been reported that 1000 rads stimulates the cytotoxic activity of NK cells, whereas a higher dose (3000 rads) completely destroys the NK activity of these cells.^{11,12} It has been suggested that the radiation sensitivity of NK cells is controlled by X-linked codominant genes. MRL mice spontaneously develop massive nonneoplastic T cell proliferation and autoimmune disease, which kills 50% of the mice by 5–6 months of age. However, the total lymphoid irradiation (3400 rads) given at the age of 3 months (time of clinical onset of disease) produced 100% survival at the age of 9 months, whereas the whole-body irradiation (300 rads) produced 82% survival.¹³ By 9 months of age, 92% of the nonirradiated mice died. It was found¹³ that after 6 months of postirradiation, the irradiated animals had normal suppressor T cell function (Con A induced); the helper T cell activity was lower than the nonirradiated control.¹³

High-dose fractionated total lymphoid irradiation allows the intake of allogeneic bone marrow in animals without clinical graft-vs.-host disease (GVHD).^{14,15} It has been suggested¹⁶ that donor-type lymphocytes in long-term total lymphoid irradiated chimeras are specifically tolerant to the tissues of the host. Therefore, the lack of GVHD may not be dependent upon the continued presence of suppressor cells of host origin or on chronic immunodeficiency of the donor-type cell.¹⁶

Ionizing radiation is routinely¹² used in organ transplantation in order to prevent rejection. In spite of this, rejection of transplanted organs does occur.

Three methods of irradiation are used in organ transplantation: whole-body, local, and extracorporeal. In clinical practice, whole-body irradiation has produced considerable mortality.¹² Local irradiation is also widely used. Extracorporeal irradiation of the blood prolonged a skin homograft in the calf.¹⁸ Ionizing radiation has helped in delaying the appearance of the GVHD reaction.

The effect of low doses (2–10 rads) on the expression of human T-lymphocyte-specific CD₂ antigen (sheep erythrocyte rosette receptor) was studied by inserting human CD₂ genes in Chinese hamster ovary (CHO) cells in culture. The CD₂ expression was not affected by low doses of X-irradiation (2–6 rads or 2–6 cGy); however, after 10 rads, 50% of the cells lost the CD₂ phenotype.³² CD₂, in the cell surface glycoprotein of 50–55 kDa is involved in lymphocyte differentiation as well as T cell activation and immune response. Lethal X-irradiation of

female mice followed by bone marrow reconstitution appeared to alleviate nonspecific immune effector mechanisms that augment host resistance to mastocytoma.³³

VI. RADIATION-PRODUCED CHIMERAS

Lethally irradiated animals can be made to survive by virtue of injections of allogeneic bone marrow cells. These survivors are referred to as chimeras, because the organisms produce cells of more than one genotype. The existence of chimerism indicates that the immunity response of the irradiated animals is completely suppressed. Radiation chimeras are capable of defense reactions.

A. BEHAVIOR OF CHIMERAS TO GRAFTS

Lethally irradiated animals protected by the administration of allogeneic or xenogeneic hematopoietic cells tolerate the skin or neoplastic cells of a donor.¹⁹ It has been shown¹ that the regenerating lymphoid tissue of radiation chimeras is of the donor's type.

B. ANTIBODY SYNTHESIS IN CHIMERAS

Whether the antibody synthesis in radiation chimeras is due to recipient or donor cells, or both, cannot be ascertained. For example, in some cases serum proteins in radiation chimeras appear to be of the donor's type, and in others they are of the recipient's type.¹

There are three different types of chimeras among lethally irradiated mice that received rat bone marrow transplantation:

1. Xenogeneic chimeras, in which the peripheral blood contained only rat erythrocytes and granulocytes. These animals accept rat skin graft permanently.
2. Individuals with blood cells of both mice and rats. These animals show some deterioration of rat skin graft.
3. Individuals with only mice blood cells, who therefore reject rat skin graft.

C. DELAYED-DEATH PHENOMENON IN CHIMERAS

Cases of delayed death (secondary disease) are frequent among allogeneic and xenogeneic chimeras. The first symptoms of secondary disease appear after the period of radiation mortality, which is during the fourth or fifth week after bone marrow transplantation. They consist chiefly of emaciation, diarrhea, and skin lesion. Weight loss occurs after a recovery of the postirradiation decrease of body weight and, therefore, is referred to as secondary disease. The secondary weight loss is accompanied by the excretion of unusually large amounts of loose wet feces, the color of which is lighter than normal. The peak of this secondary mortality lies between the 13th and 19th weeks after irradiation.¹ The mortality may be 100%. The skin lesions include loss of hair, ruffled appearance of hair, abrasions, and ulceration. Generally, human chimeras die; but occasionally, animal chimeras do not die. The exact reasons for this are unknown.

D. INCIDENCE OF LEUKEMIA IN CHIMERAS

When mice with a transplanted myeloid leukemia were irradiated with 550 R and received allogeneic spleen and bone marrow cells, the incidence of leukemia decreased²⁰ as a function of time after splenectomy. Control animals injected with syngeneic bone marrow, and then splenectomized, showed 100% incidence of leukemia. The spleen of these animals transferred leukemia in 100% of the secondary recipients, whereas the spleen of radiation chimeras produced no leukemia if the spleen was removed 5 days after treatment. It was suggested that the leukemic cells are eliminated by a GVHD reaction.

VII. RADIOSensitivity OF MICE AND DEVELOPMENT OF AUTOIMMUNE DISEASE AND NEOPLASIA

Young New Zealand Black (NZB) mice develop a high incidence of autoimmune disease and neoplasia. The $^{30}\text{LD}_{50}$ value for NZB mice with low immunity response was only 543 R.²¹ The radiation resistance of young NZB mice, combined with previous observations of their immunologic hyperresponsiveness, supports the concept that NZB mice possess an unusually large pool of hematopoietic stem cells — an abnormality that may predispose them to the development of autoimmune disease and neoplasia. It is presumed that there may be an enhanced opportunity or the triggering of autoantibody formation as a consequence of an increased availability of potentially responsive target cells.

VIII. SUMMARY AND COMMENTS

It is now well established that radiation suppresses the immunity response of whole organisms, either by inhibiting innate immunity or by inhibiting antibody formation. The effect of radiation on antibody formation depends upon whether the organisms have been in previous contact with the antigen (secondary response) or whether they are in contact for the first time (primary response). The extent of decrease in the primary response is associated with radiation dose, time of antigenic stimulus, and nature of antigen. Some workers have claimed that the primary response is more radiosensitive than the secondary response. However, others have regarded the secondary response as a sensitive phase of the immunological reaction. The expression of CD₂ was lost in 50% of Chinese hamster ovary cells that were inserted with CD₂ antigen gene after 10 rads.

Among various types of lymphocytes, B cells and unprimed T cells are very radiosensitive, whereas macrophages, carrier-primed T cells, and plasma cells are radioresistant. Radiation dose of 1000 rads stimulates the cytotoxic activity of natural killer (NK) cells, whereas higher doses reduce it. High-dose fractionated total lymphoid irradiation allows the intake of allogeneic bone marrow without GVHD. Radiation has been used in suppressing the immunological reaction after organ transplantation. Lethal X-irradiation of female mice followed by syngeneic bone marrow transplantation activates nonspecific immune effector mechanisms, which induce resistance to mastocytoma tumor.

When lethally irradiated individuals received allogeneic or xenogeneic bone marrow cells, they survived. These individuals are referred to as radiation chimeras, because they produce cells of more than one genotype. The chimeras may accept the allogeneic or xenogeneic grafts, because they carry donor cells. Generally, chimeras die; however, occasionally, death does not occur in animals. The mechanism of such a phenomenon is unknown. The incidence of leukemia in chimeras is markedly reduced. Young NZB mice that develop a high incidence of autoimmune disease and neoplasia are highly radioresistant. It has been suggested that an unusually large pool of hematopoietic stem cells may account for the above diseases.

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Chapter 14

BACKGROUND, MEDICAL, AND COMMERCIAL SOURCES

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I. INTRODUCTION

There are three major sources of background radiation: (1) terrestrial radiation due to the presence of naturally occurring radionuclides in the soil and earth, (2) cosmic radiation arising from outer space, and (3) naturally occurring radionuclides deposited in the body.

II. TERRESTRIAL RADIATION

The rate at which a person is exposed to natural background radiation is a function of the person's geographic location and living habits. The BEIR report estimates that the average dose-equivalent rate to the U.S. population from terrestrial sources is about 40 mrem/year (disregarding structural shielding). If one considers the doses to the internal organs, this value can be reduced by 40% (20% due to structural shielding provided by the building and 20% due to shielding provided by outer tissues of the body).

III. COSMIC RADIATION

Cosmic radiation includes high-energy primary particles arising from outer space that interact with matter in the earth's atmosphere and generate less-energetic secondary particles.

Thus, the atmosphere of the earth acts as a shield for high-energy radiation arising in outer space. The cosmic radiation dose-equivalent rate increases with altitude. For example, the dose rate per year at 1800 m is about double that at sea level.¹ Cosmic radiation also interacts with the earth's magnetic field, which also varies from one region of the earth to another. In addition, the cosmic radiation dose rate varies due to solar modulation.¹ It has been estimated that the variation in the cosmic radiation dose rate due to variations in the magnetic field and solar modulation is less than 10%.² The average dose rate to the U.S. population from cosmic radiation is about 31 mrem/year (disregarding shielding).² This value can be reduced by about 10% to account for the fact that people spend a large part of their time indoors, protected by the structural shielding of buildings.¹

IV. NATURALLY OCCURRING RADIOISOTOPES DEPOSITED IN THE BODY

The primary sources of these radionuclides are the atmosphere, food, or water. They include radioisotopes of lead, polonium, bismuth, radium, radon, potassium, carbon, hydrogen, uranium, and thorium, as well as a dozen or more extraterrestrially produced radionuclides.¹ The average dose rate depends upon the organ and varies from 24 mrem/year to bone marrow to 60 mrem/year to the bone surface.¹

The average dose rate to various organs from background radiation has been estimated to be as follows:¹

	mrem/year
Gonads	80
Bone marrow	80
GI tract	80
Lung	180–530

V. MEDICAL EXPOSURE

In 1970, the Bureau of Radiological Health (BRH) estimated that 65% of the U.S. population was exposed to medical exposures of radiation. The distribution was as follows:¹

Radiographic procedures	75 million
Dental diagnosis	59 million
Fluoroscopy	9 million
X-ray therapy	0.4 × 1 million

The BRH has estimated that the average absorbed dose rate for the bone marrow of the adult U.S. population from medical X-rays was 83 mrad/year in 1964 and 103 mrad/year in 1970 (Table 14.1).³ It has been estimated that medical radiographic procedures contribute about 77% of the total, and fluoroscopic and dental examinations contribute about 20% and 3%, respectively.

VI. OCCUPATIONAL EXPOSURE

The Environmental Protection Agency estimated that the mean annual dose to persons (195,000) exposed in the operation of medical X-ray equipment, and to persons (171,000) exposed in the operation of dental X-ray equipment, was 320 and 125 mrem, respectively. The estimate for dental workers was only 50 rem. The estimated mean annual radiation dose from

TABLE 14.1
Dose Estimates to Bone Marrow
after a Single Diagnostic Procedure

	mrad
Chest, radiographic	10
Thoracic spine	247
Upper GI series	535
Barium enema	875
IVP, lumbosacral spine	450
Pelvimetry	595
Dental	9.4

From Shleien, B., Tucker, T.T., and Johnson, D.W., Publ. (FDA) 77-8013, Department of Health, Education and Welfare, Washington, D.C., 1977.

diagnostic medical radionuclide and radium workers is about 375 mrem/year, radiopharmaceutical administrations are 189 mrad for the whole body, 242 mrad to the gonads, and 292 mrad to the bone marrow.⁴

VII. ATMOSPHERIC NUCLEAR TEST

From 1950 to 1960, when extensive atmospheric nuclear testings were conducted, large quantities of radioactive substances were distributed to the environment throughout the world in the form of fallout.¹ Although many of the radioactive substances may have decayed, those remaining are going to be a continuous source of exposure for some tissues. The projected annual average whole-body dose-equivalent rate for the U.S. population from these sources is about 4–5 mrem/year.

VIII. COMMERCIAL EXPOSURE

The average exposure from commercial radiation sources (power reactors, industrial radiography, fuel processing and fabrication, and processing and distribution of by-product materials) from 1973 to 1976 contributed 0.63–0.74 mrem per person per year.

The annual whole-body estimated dose rate to persons (4400) who operate electron microscopes is about 50–200 mrem.⁵ The exposure rate to the U.S. population from commercial and industrial products (television sets, luminous-dial watches, airport luggage X-ray inspection systems, dental prostheses, smoke detectors, high-voltage vacuum switches, electron microscopes, static eliminators, cardiac pacemakers, tobacco products, fossil fuels, and building materials) is about 4–5 mrem/year.⁵

The average dose rate at an altitude of 9.47 km is about 0.2 mrem/hr.¹

IX. SUMMARY AND COMMENTS

There are three major sources of background radiation: terrestrial radiation (40 mrem/year), cosmic radiation (31 mrem/year), and naturally occurring radioisotopes inside the body (24 mrem/year to bone marrow to 60 mrem/year to the surface of bone). These estimates do not

account for structural shielding. The early atmospheric nuclear tests contribute only 4–5 mrem/year. Commercial sources contribute less than 1 mrad/year. The average background radiation at sea level is about 100 mrem/year, and at a higher elevation (Denver, 5280 feet), it is about 150–200 mrem/year. Medical exposure contributes the most to individual radiation exposure. However, this amount varies from one country to another, and from one time to another within the same country. In the U.S., the medical exposure increased from 83 mrad/year in 1964 to 103 mrad/year in 1970. Occupational exposure also contributes significantly to the individual radiation exposure: individuals handling medical X-ray equipment get about 325 mrem/year; individuals handling dental X-ray equipment get about 125 mrem/year.

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Chapter 15

RADIATION INJURIES TO HUMAN FETUSES

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I. INTRODUCTION

Developing organisms constitute a highly dynamic system in which rapid cell proliferation, cell migration, and cell differentiation occur. Therefore, it is expected that the radiation response of the embryo as a whole, or of specific tissue, would markedly differ depending upon the stage

TABLE 15.1
Dose Distribution After a Pregnant
Woman Received 5 mCi of ^{131}I during
14th to 15th Week of Gestation

Age of Fetus (weeks)	Total Dose (rads)
14-15	6-8 (whole-body)
22	6500 (thyroid dose)
11 ^a	5 (whole-body)

* Thyroid has not matured fully.

Data from Dyer, N.C., Brill, A.B., Glasser, S.R., and Gross, B.A., *Am. J. Obstet. Gynecol.*, 103, 290, 1969.

of development. Many animal studies suggest that radiation-induced changes are similar in different species when they are irradiated at an equivalent stage of development.^{1,2}

II. DOSE ESTIMATE FROM DIAGNOSTIC PROCEDURES

Many studies have shown³⁻⁶ that low radiation doses are harmful to the human fetus. The CNS and optic tissues are highly radiosensitive, and small doses (5-10 rads) may cause abnormalities in these organs. Data obtained from other mammalian fetuses support the above dose-effect relationship. The extent of damage depends upon total dose, dose rate, LET (X- or γ -rays) vs. protons, neutrons, or α particles, and mode of radiation delivery (single dose vs. fractionated dose). The type and number of abnormalities may depend upon the age of the fetus and mode of radiation delivery. Most of the human data are based on the analysis of pregnant women of Hiroshima and Nagasaki who were exposed to the atomic bomb in 1945, or of women who received a radiation dose during diagnostic or therapeutic radiation procedures.

Before discussing the dose-effect relationship, it may be pertinent to describe the gonadal and fetus dose after diagnostic irradiation. It has been estimated⁵ that a gonadal dose during a single stay in the hospital could exceed 10 R. The dose distribution after a therapeutic dose of ^{131}I is shown in Table 15.1. The dose estimates of several other medical procedures have been described in Chapter 14.

III. RADIOSENSITIVE PERIODS DURING FETUS GROWTH

The most sensitive period in the etiology of a cell is during its transformation from the embryonic state to the adult state, whether it be a neuroblast, myoblast, or erythroblast. In humans, such transformation occurs between days 18 and 38 of gestation. Therefore, during this period, irradiation of the fetus causes the highest incidence of congenital anomalies. It is also clear that, during this period, a fractionated dose would produce a greater number of organ abnormalities than a single dose, because a great variety of formative cells would be exposed to radiation; and, therefore, more organ primordia would be damaged at their critical stage. It should be pointed out that this is the period during which neither the woman nor her clinician might suspect a pregnancy. Figure 15.1 shows the sensitivity of various stages of pregnancy to gross anomalies produced when human embryos are irradiated between days 18 and 45 of gestation. The first 3 weeks of human embryonic development is the most radiosensitive period, on the criterion of lethality. Cells during this period are undifferentiated and are rapidly

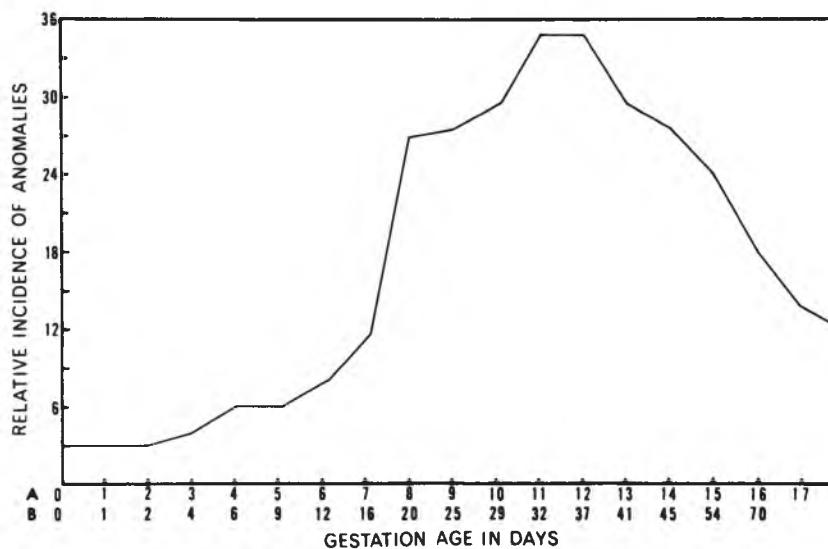


FIGURE 15.1. Teratogenesis in mice and man due to X-irradiation. If one plots the number of congenital anomalies produced in mice by X-irradiation of the embryo and fetus at various gestation ages, it is evident that the peak incidence of such anomalies among the survivors occurs when irradiation is carried out at days 11 and 12. This corresponds to days 32 to 37 for human embryos, at the time when rubella and thalidomide are believed to have their most damaging effect. (A) Mouse; (B) human. In humans the following events occur as a function of time: fertilization, 0 days; one-cell stage, 1 day; 2 to 8 cells, 2 days; morula, 4 days; implantation, 6 days; gastrulation, 9 days; early neurogenesis, 16 days; neurogenesis, 20 days; early organogenesis, 25 days; organogenesis, 29 days; skeletal differentiation, 32 days; sense organ differentiation, 41 days; hematopoiesis, 45 days; and gonad differentiation, 70 days. (From Rugh, R., *Radiology*, 9, 443, 1971. With permission.)

proliferating. Depending upon the dose, the irradiated cells could either die or recover. The recovered cells may undergo normal differentiation and maturation; therefore, radiation damage may not be reflected in terms of organ abnormalities. However, these cells may carry functional damage or mutational changes that may not become manifest during the F_1 generation. The $^{30}LD_{50}$ for the embryo observed at term increases with the gestational age at exposure (Table 15.2).^{10,11} It can be seen that the exposure required to produce 100% lethality increases as a function of gestation age. In rodents, fetus doses of 5–15 rads are known to increase lethality among irradiated zygotes and defects of the CNS, but gross congenital anomalies are rare. Radiation-induced congenital anomalies are highest during days 18–45 in humans, but sensitivity to lethality is less during this period. A dose of 200 rads causes 100% lethality in the rat when irradiated on day 9 of gestation. A dose of 510 rads before 18 weeks of gestation causes 100% mortality in the human fetus.

After the organs become differentiated and are largely involved in further growth and maturation, susceptibility to radiation-induced congenital anomalies or to the lethal effect is greatly reduced; and the effects of radiation are largely expressed as pathologic damage to the tissues, organs, and associated functional impairment. These may become apparent or detectable after varying periods of time during life. Irradiation of the fetus at any time can cause some loss of neural connections, resulting in functional deficiencies.

IV. EFFECT OF IRRADIATION ON EMBRYOS DURING THE PREIMPLANTATION PERIOD

This period includes the cleavage, morula, and blastocyst stages of the embryo. The embryo is very sensitive to radiation and can either die or survive after exposure, depending

TABLE 15.2
The Effect of Irradiation on the
Mammalian Embryo

Gestational Day in the Rat	LD₅₀ of Embryo Observed at Term (R)
0-8	30-120
9	120
10	160
11	210
12	240
Adult rat	600

A portion of the data was summarized from Brent, R.L.,
Clin. Obstet. Gynecol., 3, 928, 1960.

upon the dose. Those embryos that survive do not show any abnormality with respect to morphology, size, short- and long-term survival, and reproductive ability.¹²⁻¹⁴ Diagnostic irradiation of the human female pelvis before an early pregnancy does occur. In human development, the first cleavage occurs as late as 36 hr after fertilization. The effect of irradiation on the precleavage embryo has been studied in mice.¹⁵ There was a significant reduction in the number of implantations when 25 or 50 R was applied to the 6- to 8-hr-old zygotes (Table 15.3). The percentage of normal surviving implantations, following exposure (10-50 R) at 7 hr postconception, significantly decreased. At a higher dose (50 R), the drop in normal fetuses occurred when the implanted zygote was irradiated as early as 2 hr postconception (Table 15.4). There was no significant difference in the percentage of dead and anomalous animals. Therefore, the differences in the percentage of normal fetuses must be attributed to differences in resorptions or very early death. It has been recommended¹⁶ that X-ray exposure of the human pelvis should be restricted to the first 9 or 10 days after the onset of menstruation, because conception normally occurs at about day 14. Therefore, exposure of the pelvis of women of reproductive age during the third week to even 10 R could be deleterious. However, this issue has become controversial. Some institutions follow this recommendation, whereas others do not. This issue will be discussed at the end of this chapter.

It has been reported¹⁷ that when mice are exposed during the preimplantation period (soon after sperm entry), sex chromosome loss occurs — which may result in XO females (Turner's syndrome in humans). The frequency of this effect in mice is about 4% after an exposure of 100 R of X-rays.¹⁷ Loss of any chromosome other than X or Y may contribute to early death.

TABLE 15.3
Effect of Irradiation on the
Implantation of the Zygote*

Age (hr)	Average % Normal Implantation			
	Control	10 R	25 R	50 R
2	85.0	83	81	76
6	85.0	79	71	61
7	85.0	76	71	59
24	85.0	87	77	83

* Data are presented in a simplified form and were taken from Rugh, R., Wohlfomm, M., and Verma, A., *Radiat. Res.*, 37, 401, 1969.

TABLE 15.4
Effect of Irradiation on Zygotes Irradiated at 7 hr Postconcentration^a

Age Fetuses Lost	Number of Implantations	Number of Normal Implantations	Number of Abnormal Implantations
Control	596	504	92
7 hr (10 R)	583	449	134
7 hr (25 R)	583	401	182
7 hr (50 R)	583	308	275
2 hr (50 R)	581	441	140

^a Data are presented in a simplified form and were taken from Rugh, R., Wohlfomm, M., and Verma, A., *Radiat. Res.*, 37, 401, 1969.

Albino female mice were irradiated with neutrons (7 MeV) or X-rays when embryos were at the early zygote stage. Mortality (Figure 15.2) and malformed fetuses (Figure 15.3) were determined at 19 days of gestation. The survival of fetuses decreased exponentially with dose, and RBE of neutrons was 2.3. On the other hand, the number of malformed fetuses increased with a linear-quadratic function of neutron or X-ray dose (Figure 15.3), and RBE of neutrons varied from 2 to 2.8. These results suggest that radiation exposure to early zygote could be more complex than the simple all-or-none response.⁷⁰

V. EFFECT OF IRRADIATION ON EMBRYOS AFTER IMPLANTATION

Table 15.5 describes some of the effects of irradiation on the embryo after implantation. It is apparent from several studies¹⁸⁻²⁶ that a few rads of irradiation during the sensitive period (period of organogenesis) could produce deleterious effects. Some studies have shown^{27,28}

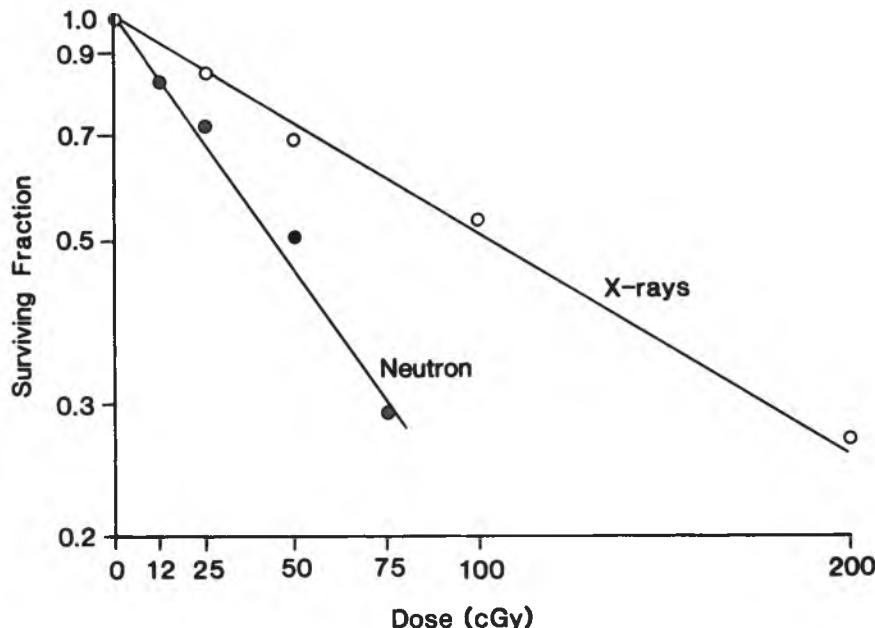


FIGURE 15.2. Fraction of surviving fetuses at gestation day 19 as a function of the neutron or X-ray dose. (From Pampfer et al., *Teratology*, 37, 599, 1988. With permission.)

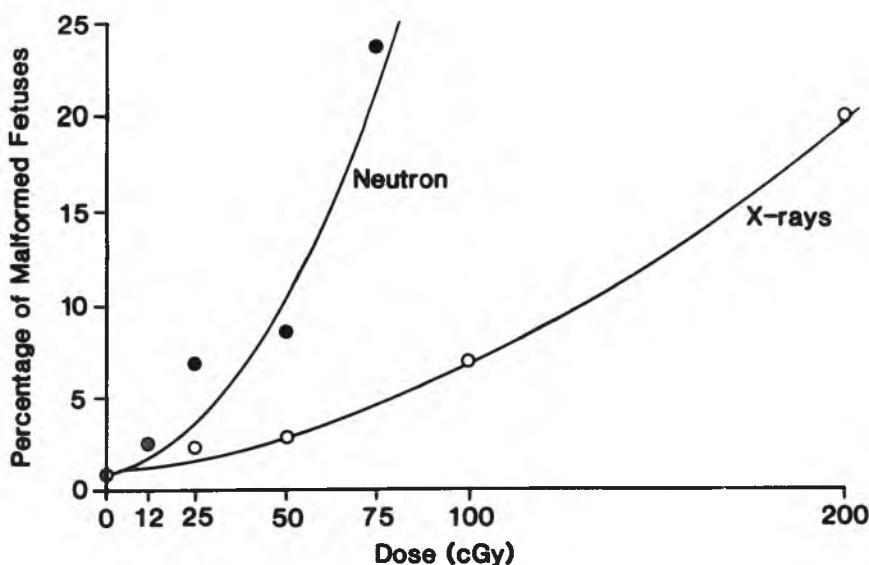


FIGURE 15.3. Incidence of externally malformed fetuses at gestation day 19 as a function of the neutron or X-ray dose. (From Pampfer et al., *Teratology*, 37, 601, 1988. With permission.)

that a dose of less than 10 rads to the human fetus does not produce gross developmental defects. However, another study has found a significant defect in the central nervous system among children who received diagnostic doses of radiation (400 mrad) *in utero*.²⁹ A review by Mole³⁰ suggested that prenatal irradiation during the fourth month of pregnancy would produce an average risk of 0 to 1 case (cancer and malformation) per 1000 irradiated persons exposed to 1 rad. This estimate is 30 times less than what occurs naturally. This suggestion of Mole is inconsistent with several human epidemiological studies.

VI. EFFECT OF PROTRACTED AND FRACTIONATED DOSES

Protraction of radiation doses (continuous radiation at low dose rate) diminishes the incidence of gross abnormality.^{31,32} This may be due to the fact that the threshold doses

TABLE 15.5
Effects of Irradiation During Organogenesis in Animals

Exposure	Effects	Reference
25 R (mouse)	Skeletal defect	18
<10 R (mouse)	Mitotic delay in telencephalon region of brain	19
5 rads (mouse)	Kills 50% oocyte	20
<5 rads (human, survivors of atomic bomb, Hiroshima)	11% microcephaly	21
5–10 rads (human)	17% microcephaly	21
0.0086 R/min, 12.4 R/day (rat)	Reduced reproductive capacity in females	22
3 rads/day (rat)	Reduced organ weight	23, 24
0.3 rads/day (rat)	Decreased brain weight in F ₂ generation	25

Note: Period of organogenesis: 7.5–12.5 days in mice, 8.5–13.5 days in rats, 14–50 days in humans. Data were summarized from the BEIR report¹ and the work of Russell.¹⁸

received within the sensitive period may be less than that needed to produce detectable injury.²² On the other hand, fractionation of single doses is about 1.5 times more effective than continuous irradiation delivered during the same interval.³³

VII. MODIFICATION OF THE EFFECT OF IRRADIATION

The nonteratogenic doses of caffeine and chloroquine enhance X-ray-induced birth defects.³⁴ The interaction of radiation with other agents that modify the effect of irradiation has not been studied during development. This study is very important because this would help in assessing the potential hazard of radiation alone, as well as in combination with other modifying agents.

Irradiation of mice at 8 days of gestation with 200 R produces the highest incidence of readily detectable nervous system abnormalities such as exencephalia. *S*-2-Aminoethylisothiourea-dihydrobromide (AET) is a good radioprotective agent for rodents. AET, when given to pregnant mice 15 min before irradiation, afforded no protection to the fetus in terms of deaths, resorption, and anomalies;³⁵ however, radioprotective agents such as cysteamine increased the survival slightly when given on day 14.5 of gestation. In those rats that appear normal following prenatal irradiation (50–185 R) and survive to adulthood, learning deficiencies are usually observed; however, this can be prevented by the administration of cysteamine (100 mg/kg of body weight) 15 min before irradiation.

VIII. EFFECT ON NERVOUS TISSUE

For the criteria of brain abnormalities, neuroblasts are more sensitive than neuroectoderm, whereas neurons are highly radioresistant. X-irradiation of human fetuses at any time before the completion of neurogenesis induces severe CNS anomalies. Among CNS defects, microcephaly (often associated with mental retardation) is most common. This defect may not be apparent upon histological examination, regardless of the degree of neuronal loss. Some sixty-four percent of the children exposed *in utero* to atomic bomb radiation within 1200 m, and who appeared normal at delivery, showed microcephaly with mental retardation by 4.5 years of age. Microcephaly is particularly associated with exposure during early stages of pregnancy. It has been reported³⁶ that when fetuses were exposed to radiation during 4–13 weeks of gestation, the incidence of microcephaly was 28%, whereas it was only 7% when irradiation was given after 13 weeks of gestation.

Table 15.6 describes the incidence of microcephaly among the children of Hiroshima who were exposed to atomic bomb radiation during the most sensitive fetal phase (6–11 weeks).³⁷ There was no significant increase in microcephaly below 150 rads among children of Nagasaki who were exposed during the same period of gestation.³⁶ However, a recent study⁷³ shows that 11% of the fetuses of under 18 weeks of age who were exposed to 0.10–0.19 Gy (10–19 rads) developed microcephaly.⁷³ This value is similar to that reported earlier.³⁶ This may be due to differences in the quality of radiation. Another report³⁸ estimated the damage to groups of children who were exposed to the atomic bomb at Hiroshima. The number of brain abnormalities was high. BEIR V, 1990 has proposed that a threshold for mental retardation may lie between 0.2 and 0.4 Gy (20–40 rads).

Pelvimeteric irradiation of pregnant women before 5 months of gestation caused 20% of their children to be born with mental deficiency. Among A-bomb survivors exposed at 8–15 weeks of gestation, the IQ was 21–29 points per Gy.⁷³ Based on a review of Russian literature, there is a suggestion that the nervous tissue function may be altered by doses as low as 100

TABLE 15.6
Effect of Irradiation During the Most
Sensitive Period (6–11 Weeks) on the
Incidence of Microcephaly

Air Dose	Incidence of Microcephaly (%)
1–9 rads ^a	11
10–19 rads ^b	17
20–29 rads	30
30–49 rads	40
50–99 rads	70
>100 rads	100
0 dose	4

^a 1–9 rads (air dose) = average fetus doses, 1.3 rads plus 0.1 rad neutron.

^b 10–19 rads (air dose) = average fetus doses, 5–3 rads of γ -radiation plus 0.35 rads of neutrons.

Data were summarized from the BEIR report.^{1,37}

mrad.^{39,40} The incidence of seizure was highest among subjects who were exposed to radiation during 8–15 weeks of gestation with doses higher than 0.10 Gy (10 rads). The severity of seizure increased linearly as a function of fetal doses.⁷⁴ No seizure was observed among subjects who were exposed to radiation during 0–7 weeks of gestation with doses higher than 0.10 Gy (10 rads).

The effect of irradiation on the fetus of the rodent has been studied extensively in relation to the CNS. A single dose of 10–40 R produced marked changes in the developing brain. It diminished the formation of cytoplasmic basophilic materials in the nerve cells and inhibited their growth. An exposure of 20–40 R (single exposure) caused permanent alteration of individual nerve cells and also interfered with their organization into a neuronal assembly, such as the layer of cerebral cortex. Such exposures also produced an irregular branching of dendrites.⁴¹ An exposure of 10–40 R (single exposure) caused permanent alteration of individual nerve cells and also interfered with their organization into a neuronal assembly, such as the layer of cerebral cortex. Such exposures also produced an irregular branching of dendrites.⁴¹ An exposure of 10–40 R to the fetus may damage the cerebral cortex and Purkinje cell morphology in rats. Prenatal exposure to radiation also affects the functional, morphological, behavioral, and biochemical development of the brain. Pregnant rats receiving 25 or 50 R of X-rays to the pelvic region on the 14th day of gestation were allowed to deliver and rear their offspring. Carbonic anhydrase activity was suppressed in the medulla and spinal cord of male offspring. Activity of acetylcholinesterase was decreased in the caudate nucleus of all ages up to 80 days in both male and female offspring of the irradiated mother.⁴² An exposure of 100 R on the 9th day of gestation changed EEG activity, whereas an exposure of 185 R caused hyperactivity to visual stimuli. An exposure of 24 R during gestation at 25 days decreased the performance capacity of the rat.

Exposure of 15 R of X-rays on day 0.5 or day 1.5 of gestation (at the one- and two-cell stage of embryonic development) caused exencephaly in mice. Exposures of 12.5–50 R administered during the period of neurogenesis produced brain hernias, hydrocephalus, anacephaly, spinal cord abnormalities, ocular defects, and anophthalmia. An exposure of 10–40 R is sufficient to alter the morphological development of the cortex. Exposures of 30–40 R may lead to distortion of Purkinje cells in rats exposed on the day after birth.

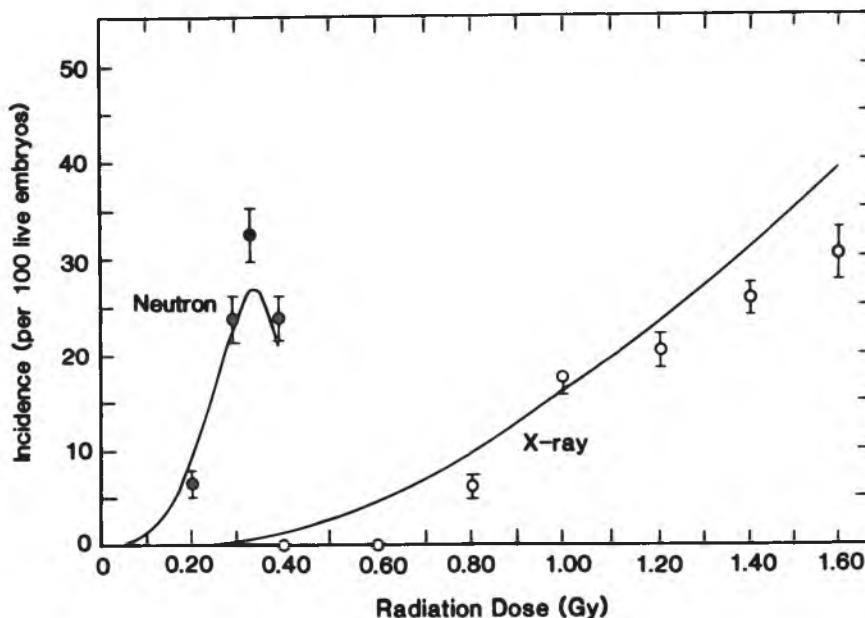


FIGURE 15.4. Incidence of exencephalia in live embryos as a function of radiation doses. Irradiation was on gestation day 8. Figure was redrawn for simplicity. (From Friedberg et al., *Int. J. Radiat. Biol.*, 52, 227, 1987. With permission.)

The incidence of exencephalia in live mouse embryos irradiated at 8 days of gestation with neutrons increased and then declined (Figure 15.4). In contrast, X-ray-induced exencephalia showed only an increase with dose.⁷⁵ The decline in incidence of exencephalia in neutron-irradiated embryos was due to increased mortality *in utero*.

Abnormal neurological signs (ataxia, seizures, gait defect, forced circling, righting) have been observed in rats exposed to 20 or 100 R on gestation day 10, and to 50 or 185 R on gestation day 15. An exposure to 50–100 R impaired the motor activity. Increased frequency of endogenous convulsive seizures has been reported in mice that were exposed to radiation (150 mrad/day for a total dose of 1.5–5 rads) between days 23 and 30 of gestation.⁴³ Abnormal behavior also has been reported after exposure.

IX. EFFECT ON THE EYE

Among eye defects, anophthalmia and microphthalmia are common gross responses in rodents exposed to 100–300 R at a particular stage during fetal life. Although the developing retina is sensitive to radiation, it exhibits a remarkable capacity to reconstitute and repair the damaged area. Even after reconstitution of the retina, there is evidence of persistent damage in the form of microphthalmia. There is a progressive fall in radiosensitivity during the first week after birth as retinal cells become differentiated. A dose of 15 rads to the rat fetus produces brain and eye abnormalities.⁴⁴ The exposure of the fetus to doses of less than 5 rads may produce eye defects in the F₂ generation.⁴⁵ In another study, the incidence of anophthalmia in live mouse embryos irradiated at 8 days of gestation with neutrons increased and then declined. In contrast, X-ray-induced anophthalmia showed only an increase with dose (Figure 15.5).⁷⁵ The decline in incidence of anophthalmia in neutron-irradiated embryos was due to increased mortality *in utero*.

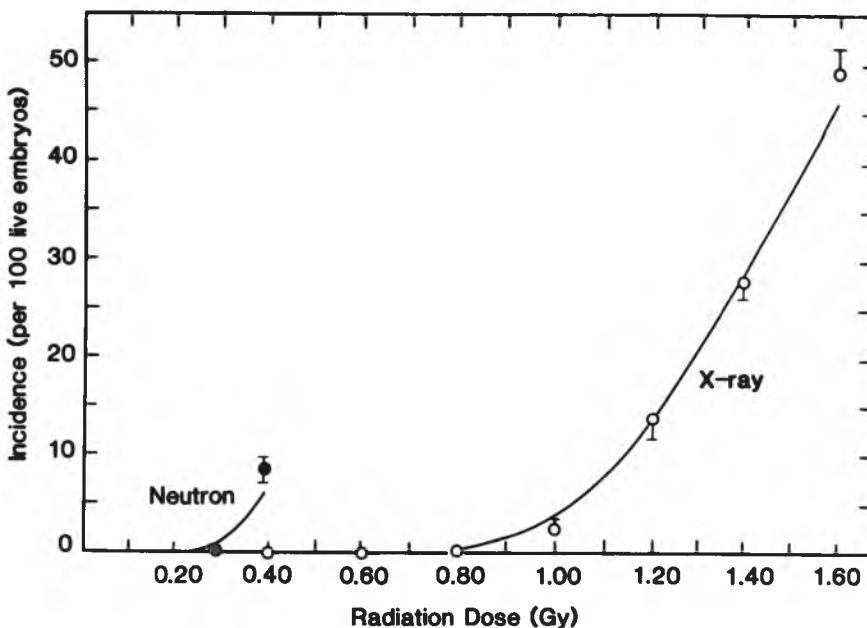


FIGURE 15.5. Incidence of anophthalmia in live embryos as a function of radiation doses. Irradiation was on gestation day 8. Figure was redrawn for simplicity. (From Friedberg et al., *Int. J. Radiat. Biol.*, 52, 230, 1987. With permission.)

X. EFFECT ON GONADS AND OTHER ORGANS

Pregnant rats were exposed to 100 R of X-rays at days 13.5, 15.5, 17.5, and 19.5 of gestation. The offspring were analyzed 100 days after birth. Quantitative studies of the population of germ cells confirmed that the radiosensitivity of the germinal elements of the female is maximal at 15.5 days of gestation. In contrast, the male germ cells became increasingly radiosensitive as pregnancy advanced (17.5–19.5 days of gestation). Severe depopulation of oocytes appeared to be associated with a slight decline in the secretory activity of the ovary. An exposure of 100 R was sufficient to cause almost complete elimination of male germ cells, but the endocrine function of the testis was not affected.⁴⁶ Female mice were irradiated on the 11th day of pregnancy with 20 and 80 R. The female offspring, at the age of 28 days, showed a reduction in oocytes amounting to between 20 and 45% in comparison with controls. The fact that the reduction was less at the age of 50 days than at 28 days indicates a repair process.⁴⁷

A pregnant woman (23 years old) with Hodgkin's disease received cyclophosphamide and radiation (fetus dose about 5–25R) during the first trimester. Her baby was delivered at 6 months of gestation, and a fetal autopsy showed an absence of all toes and a single coronary artery.⁴⁸ It is difficult to estimate whether the abnormality was produced by irradiation or chemotherapeutic agents, or both. However, irradiation-induced cardiac anomalies have been reported in experimental animals and humans.¹⁶ The irradiation of pregnant rats with 130 rads of 14.1-MeV fast neutrons on day 8 of gestation causes malformations of the heart and large vessels in 85% of fetuses.

XI. EFFECT ON INCIDENCE OF NEOPLASM

Stewart and Kneale⁶ reported that an increase in crude excess of cancer risk is directly proportional to the number of X-ray films or to fetus dose (Figure 15.6). The cancer risk was

greater when exposure was during the first trimester. They estimated that 1 rad delivered to the fetus shortly before birth would cause an increase of 300–800 deaths per million before the age of 10 years due to cancer [572 ± 133 (SE) deaths]. This corresponds to an annual absolute risk of 57 cancer deaths per million persons per rad during the period of observation of 10 years. Further analysis of these data by other investigators^{50–58} confirms the hypothesis of a linear relationship between fetal dose and the relative incidence of cancer. However, the magnitude of the effect has been questioned by another group of investigators.⁵⁸

Table 15.7 shows the dose–effect relationship. It was estimated that the typical dose to the fetus from pelvimeteric examinations during 1927 to 1956 was about 4 rads. MacMahon⁴ concluded that for children born in the northeastern U.S. in the years 1947–1954, childhood cancer mortality was about 50% higher in the X-irradiated group than in the nonirradiated one. A significant increase⁵³ in malignancy has been found even after 200–250 mrad to the human fetus (Figure 15.7). The incidence of leukemia was higher among children irradiated *in utero*; the incidence was still higher if the mother had previously experienced miscarriage or stillbirth (Table 15.8). However, in a separate series of studies, the malignancy did not show an increase among children who received an X-ray exposure of 1.5–32 rads *in vitro*; but the incidence of hemangiomas in the irradiated children increased by a factor of 2. Another study⁶⁹ failed to show an increase in the frequency of leukemia in children exposed to diagnostic radiation *in utero*. One study⁶¹ has shown that there are at least two subgroups of children (susceptible and nonsusceptible) in the population. Exposures to low-level radiation — such as diagnostic X-rays — during pregnancy produced little increase in risk of leukemia in the “nonsusceptible” subgroup. However, the same radiation exposure can increase the relative risk almost ten times in subgroups with a relatively high proportion of “susceptible” subjects, who can be identified on a probability basis from the health record of mother and child. Figure 15.8 indicates the existence of a radiation-susceptible group of children. This study further supports the experimental data that radiation-induced carcinogenesis can be modified by cocarcinogens, tumor promoters, and antitumor promoters. Recent studies suggest that diet and lifestyle factors can also affect cancer incidence.

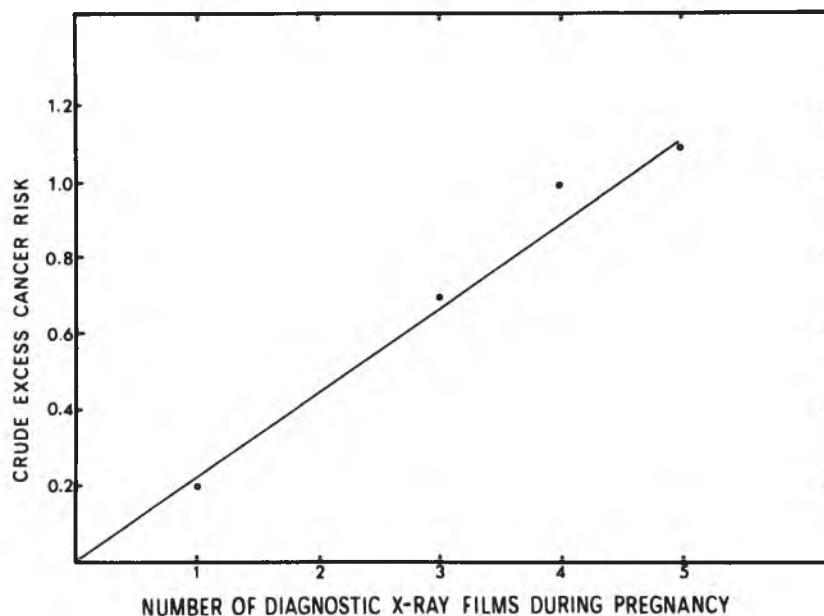


FIGURE 15.6. Crude excess of cancer risk relative to X-ray of fetus dose. (From Stewart, A. and Kneale, G.W., *Lancet*, 1, 1185, 1970. With permission.)

TABLE 15.7
Effects of Irradiation on the Human Fetus for the Criterion of Malignancy

Fetus Age	Fetus Dose	Type of Abnormality	Frequency	Reference
Shortly before birth	~1 rad	Leukemia	An increase of 300-800 deaths/ 10^6 person	6
Unspecified	1-3 rads	Leukemia	An increase of 30-50%	4
1st trimester	Diagnostic dose	Total malignancy	Irrad: control 5:1	4, 50, 51
2nd and 3rd trimester	Diagnostic dose	Total malignancy	Irrad: control 1.47:1	
Unspecified	1 rad	Tumor	1/2000	52
Unspecified	Diagnostic dose (200-250 mrad)	Neoplasm	Significant	53, 54
Twins*	Diagnostic dose	Leukemia	2.2:1	55
Singletons	Diagnostic dose	Leukemia	1.5:1	55
Twins	Diagnostic dose	Solid tumor	1.6:1	53
Singletons	Diagnostic dose	Solid tumor	1.5:1	53

* Re-analysis of data of Oxford survey.^{4,50,51}

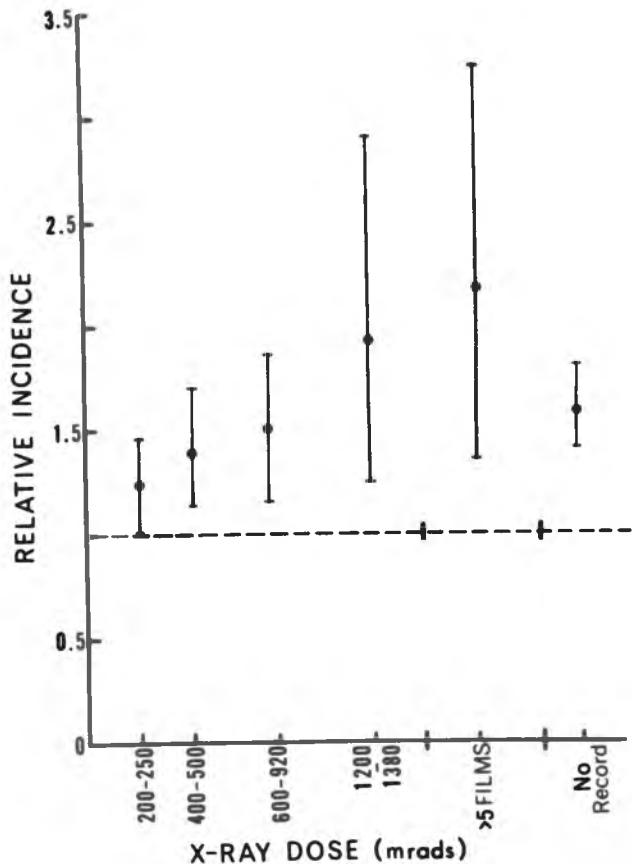


FIGURE 15.7. Relative incidence of childhood cancer following obstetric vs. no irradiation (with 95% confidence interval). From Newcombe, H.B. and McGregor, J.F., *Lancet*, 2, 1151, 1971. With permission.)

TABLE 15.8
Effect of Low Dose of Maternal Irradiation on the
Incidence of Leukemia Among Children

Female Irradiated Before Conception	Female Irradiated After Conception*	Incidence of Leukemia Risk in Children
Diagnostic X-ray		1.6 times
	Diagnostic X-ray	1.5 times
	Diagnostic X-ray and previous miscarriage	2.2 times
Diagnostic X-ray	Diagnostic X-ray	2 times

* Children of mothers who had previously experienced miscarriage or stillbirth.

Data summarized from Graham, S., Levin, L.L., Lilienfield, A.M., Schuman, L.M., Gibson, R., Dowd, J.E., and Hempelmann, L., *Natl. Cancer Inst. Monogr.*, 19, 342, 1966.

Therefore, it is important to take into consideration the effect of the interaction of radiation with environmental dietary and lifestyle factors when assessing the effect of ionizing radiation on human fetuses.

The incidence of cancer was analyzed among children exposed *in utero* who were born between the days of the atomic bombing (August 6, 1945 in Hiroshima, and August 9, 1945

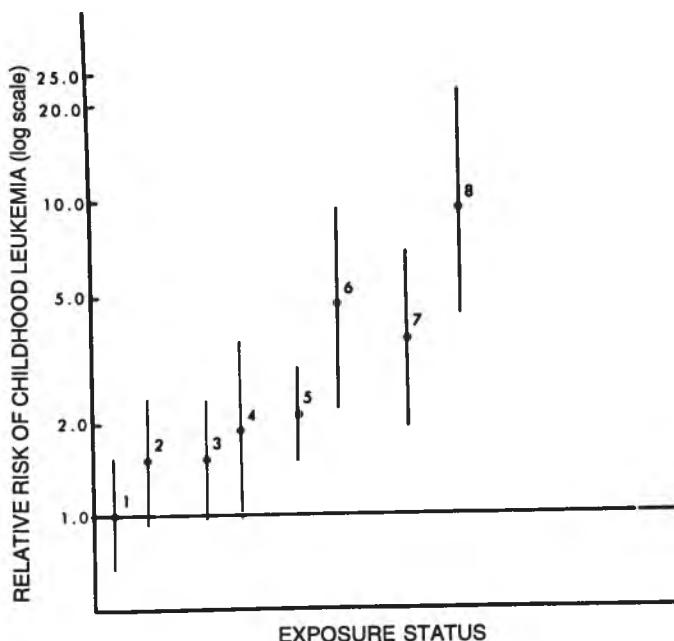


FIGURE 15.8. Approximate confidence intervals on the relative risk of childhood leukemia (age-adjusted risks in relation to children not exposed to intrauterine radiation and without report of specified childhood disease) according to exposure to intrauterine radiation and history of disease; (1, 2): no report of the specified disease; (3, 4): report of red measles or chicken pox; (5, 6): report of pneumonia, whooping cough, or dysentery; and (7, 8): report of asthma or hives. (From Bross, I.D.J. and Natarazan, N., *N. Engl. J. Med.*, 287, 107, 1972. With permission.)

TABLE 15.9
**Incidence of Cancer Among Children Who
 Were Exposed to Radiation *In Utero*^a**

Uterine Dose	Case/10 ³ Person
0	7
≥0.01 Gy (1 rads)	14
0.01 – 0.29 Gy (1–29 rads)	10
0.30 – 0.59 Gy (30–59 rads)	23
≥0.6 Gy (60 rads)	11

^a Data were summarized from a previous study.⁷¹ Relative risk of all cancers at 1 Gy (100 rads) among children exposed *in utero* was 3.77.

in Nagasaki) and May 31, 1946. The incidence of cancer during 1950–1984 increased by about 2- to 3-fold, depending upon dose⁷¹ (Table 15.9).

Persons exposed *in utero* showed an increased frequency of cancer.^{72,73} The incidence of breast cancer was higher among girls exposed to atomic bombing under 10 years of age than among those who were older at the time of bombing.⁷² Among those girls who received 0.5 Gy (50 rads) or more, 10 cases of breast cancer were observed, as compared with two expected cases.⁷³

XII. DURATION OF RISK

A recent study⁵¹ suggests that increased risk for hematopoietic tissue exists for the first 12 years of life, whereas the risk for solid tumor exists (0–14 years) but is reduced during later years (9–14 years). The BEIR¹ report estimates that the period of increased risk appears to begin at birth, lasts for 12 years for hematopoietic tumors, and largely disappears within 30 years after exposure. Solid tumors seldom appear before 10 years after irradiation, and they may continue to appear for 30 years or more after exposure.

XIII. EFFECT OF DIAGNOSTIC RADIATION RECEIVED PRIOR TO CONCEPTION

Radiation increases the risk of nondisjunction (uneven distribution of chromosomes in the daughter cells) during mitosis in a subsequent pregnancy. Table 15.10 shows that young females who had received diagnostic radiation (0.5–7 rads, gonadal dose) before conception had ten times more aneuploidy children (one excess or one deficient chromosome) than those who received no irradiation.⁶² A gonadal dose of about 5 rads to the woman before conception also produces an eye defect in subsequent children.⁶³ The estimated minimal spontaneous frequency of point mutation per generation due to naturally occurring mutation-causing agents is 2%. This mutation rate includes less than half of the gross abnormalities, such as mental defects, hematologic and endocrine defects, defects in vision and hearing, cutaneous and skeletal defects, and defects in the GI tract that occur at birth. It does not include those mutations whose effects are less drastic. A gonadal dose of 3 R, which may be received by medical exposure over 30 years, would increase the number of point mutations of all degrees by about 1%.⁶⁴

The studies on the effects of diagnostic doses of X-rays before conception are not sufficiently adequate to draw any definitive conclusion.

TABLE 15.10
Effects of Diagnostic Radiation in Young Women before Conception

	Number in Sample	Abnormalities in Children	Reference
Control	972	1 Aneuploid	62
Gonadal dose 0.5–7 rads before conception	972	10 Aneuploids (8 mongols and 2 trisomy 18 syndrome)	62
Gonadal dose 5 rads	Unknown	Eye defect	63
Gonadal exposure of 3 R		1% increase in spontaneous point mutation rate	64

XIV. CHROMOSOMAL ABERRATION

An infant who was born with multiple congenital anomalies received whole-body irradiation of 835 mrad during diagnostic procedures. When his peripheral blood was cultured and compared to the blood taken before irradiation,⁶⁰ a higher incidence of chromosomal aberration was found. Complex cytogenetic abnormalities among Japanese survivors of the atomic bomb were noted 20 years after exposure.⁶⁵

XV. RADIOISOTOPES AND THE FETUS

The fetus receives a significant radiation dose when the mother receives radioisotopes as a therapeutic or diagnostic procedure. The extent and type of damage would depend upon the physical and biological half-life of the radioisotope, and upon the pattern of organ and cellular distribution. For example, ¹³¹I concentrates in the thyroid in the ratio of 10,000:1 as compared to its concentration in other tissues, whereas ⁵⁹Fe concentrates primarily in the erythropoietic tissues. The human fetal thyroid does not take up ¹³¹I in the first 12 weeks of life, but after the 14th week increasing amounts of ¹³¹I may be taken. Dose distributions⁸ after an administration of 5 mCi of ¹³¹I to a pregnant woman during the 14th to 15th week of gestation is shown in Table 15.1. Sternglass⁶⁶ suggested that ⁹⁰Sr, resulting from tests of nuclear weapons, has killed 400,000 children in the U.S. since 1945; however, this conclusion has been disputed by Lindop and Rotulat.⁴⁵ A high incidence of neoplasms among children was reported after the administration of ⁵⁹Fe to pregnant women.⁶⁷ The estimated fetus dose was 5–15 rads. Among 634 children exposed, one leukemia and two sarcoma cases were discovered. No malignancies were reported in the control group.

XVI. COMMENTS ON THE TEN-DAY RULE

This rule states that young women of reproductive age should not be exposed to diagnostic doses of radiation during the ten-day period following the first day of the last menstrual period. This period is generally not associated with unsuspected pregnancy. Some institutions follow this rule, and others do not. Some recommend its continuation;⁶⁸ however, the International Commission on Radiological Protection⁶⁹ states,

"Because of risk of radiation injury to any embryo or fetus, the possibility of pregnancy is one of the factors to be considered in deciding whether to make a radiological examination involving the lower abdomen in a woman of reproductive capacity. Although such an examination is less likely to pose any hazard to a developing embryo if carried out during the 'ten-day' interval following the onset of menstruation, attention

should always be paid to details of radiological technique that would ensure minimization of exposure to any embryo or fetus that may be present — whether or not the woman is known to be pregnant."

If one believes that when fetuses are irradiated during the first trimester, the risk of leukemia is five times higher than the nonirradiated controls, then the ten-day rule does not appear to be valid. Therefore, a firm recommendation on this issue cannot be made. The best approach is to decide the individual problem on the basis of risk vs. benefit. If the individual is exposed to radiation exposure, the proper procedure must be followed to reduce the exposure of noninvolved areas.

XVII. SUMMARY AND COMMENTS

There is no doubt that the developing embryo is extremely sensitive to ionizing radiation; however, the effects produced are strongly dependent upon the stage of development and the mode of radiation delivery (single dose, fractionation, protraction). It also appears that the radiosensitivity of developmental stages of animals closely resembles that found in humans. Therefore, the animal data can be used to estimate the radiation hazards in humans. The BEIR report estimates that radiation exposure during the preimplantation period in the human probably produces no abnormality in survivors. This is due to the fact that the embryo maintains high plasticity during this period, and the embryo can repopulate fully even after a significant loss of cells. Radiation exposure after implantation may produce organ defects, growth retardation, functional impairment, and malignancy. The fetus is extremely sensitive to radiation for the criterion of organ defects during the period of organogenesis, which, in humans, extends for about 2–9 weeks after conception. The radiation exposure after organogenesis can produce other types of damage. Some of the radiation-induced changes in fetuses are observed at birth, whereas others may appear at a much later date. Some of the functional changes may not be measurable at the time of birth. Among organ defects, microcephaly associated with mental retardation is most pronounced after exposure of fetuses during the period of organogenesis. Data on animals and on survivors of the Hiroshima and Nagasaki atom bomb indicate that the radiation doses of 10 rads or below during organogenesis may produce brain abnormality. Exposure of the fetus at any stage of development with diagnostic doses increases the risk of childhood leukemia. The risk appears to be higher during the first trimester than during the second and third trimester. There appears to be no threshold dose for leukemia among children who received radiation *in utero*; however, for most organ defects, there appears to be a threshold dose. Lowering the dose rate decreases the damage. Neutron irradiation of zygotes or embryos *in utero* causes greater mortality and malformation than X-rays. The BEIR report suggests that "until an exposure has been clearly established below which even subtle damage does not occur, it seems prudent not to subject the abdominal area of women of childbearing age to quantities of radiation appreciably above backgrounds, unless a clear health benefit to the mother or child from such an exposure can be demonstrated."

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Chapter 16

RADIATION-INDUCED GENETIC DAMAGE

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I. INTRODUCTION

In 1927, H.J. Muller first demonstrated that X-irradiation of the sperm of the fruit fly, *Drosophila melanogaster*, induces gene mutation. Radiation is one of the many environmental agents that cause genetic defects. In addition, many spontaneous mutations may result from errors made during the replication of genetic materials. Almost all mutations are harmful to a varying degree for the organisms; however, mutations have been the basis for evolution of animal species.

Radiation causes genetic defects because of mutation in genetic materials:

1. Mutation may result from a change in nucleotides, which will alter the normal coding for amino acids in proteins. This is called *gene mutation*.
2. Mutation may result from *damage of chromosome*. The chromosomal damage may result in loss, duplication, and rearrangement of chromosomes and, thereby, genetic materials. Chromosomal damage may be caused by a single break or a double break of the chromosomes. Most (up to 90%, depending upon the experimental system) radiation-induced mutations are deletion type and are recessive in nature.

II. DOSE-RESPONSE CURVE FOR GENE MUTATION

The frequency of gene mutation increases with dose, within a certain dose range (Figure 16.1). The frequency of this type of mutation is independent of dose rate, fractionation, LET, and temperature. For low dose and low dose rate of low LET radiation, the genetic risk in the human is estimated by the linear dose-response model. The mutation due to chromosomal damage is estimated by the linear-quadratic dose model.

III. DOSE-RESPONSE CURVE FOR CHROMOSOMAL DAMAGE

The dose-response curve for a single-break effect is linear, and it resembles the dose-response curve of gene mutation (Figure 16.2). However, the dose-response curve of a double-break effect is of the sigmoid type and requires a threshold dose (Figure 16.2).

IV. MODIFICATION OF CHROMOSOMAL DAMAGE

The frequency of mutation due to a two-break effect is dependent upon the dose rate, LET, oxygenation, temperature, and radioprotective agents:

1. Effect of dose rate: the higher the dose rate, the greater the number of mutations.
2. Effect of high LET radiation: the dose-response curve of the two-break effect after low LET radiation exposure requires a threshold dose; however, this becomes linear after high LET radiation (neutron) exposure (Figure 16.3).
3. Effect of oxygen: the presence of oxygen during X-irradiation increases the frequency of chromosomal aberrations in the root tips of *Vicia faba*.¹
4. Effect of temperature: lowering the temperature during X-irradiation of *Tradescantia* microspore increases the number of chromatid aberrations.¹ This may be due to the fact that less repair of radiation damage occurs at low temperatures.
5. Effect of radioprotective agents: many radioprotective agents, such as glutathione and β -mercaptoethylamine, decrease the frequency of chromosomal damage when given before X-irradiation.¹
6. Radiation increases the frequency of an existing mutation rate.

V. TYPES OF MUTATION

A. SINGLE DOMINANT MUTATION

This type of mutation appears in the F₁ generation. Some of the diseases that are caused by a single dominant mutation include:

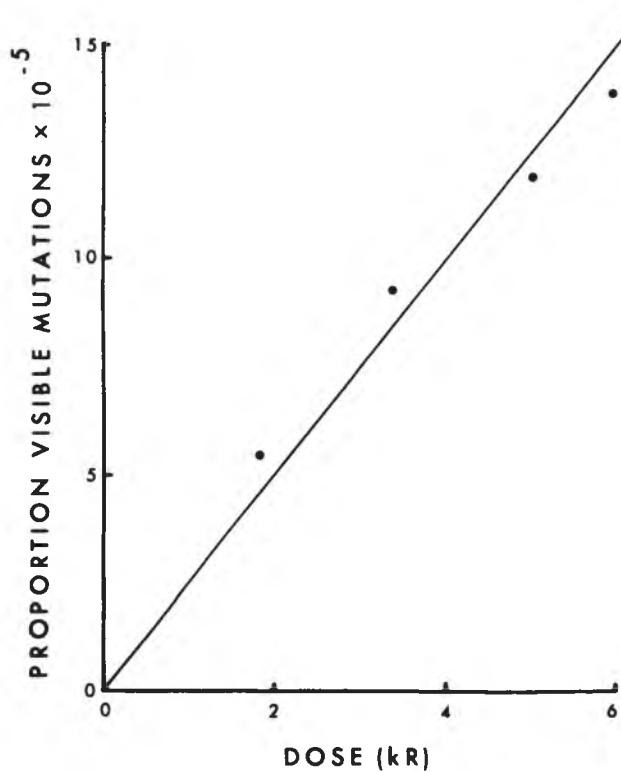


FIGURE 16.1. Linear relation of visible mutation to dose. (From Wolf, S., *Mechanics in Radiobiology*, Errera, M. and Forssberg, A., Eds., Academic Press, New York, 1961, 424. With permission.)

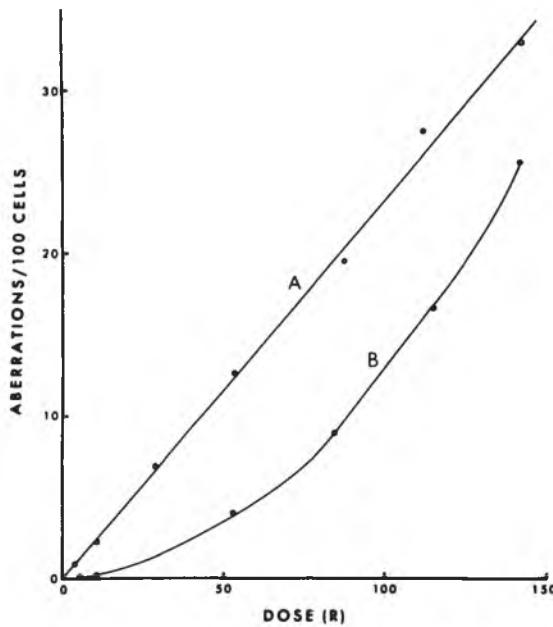


FIGURE 16.2. Dose-effect curves for aberration induced by X-rays. Curve A: one-break aberrations; curve B: two-break aberrations. (From Wolf, S., *Mechanisms in Radiobiology*, Errera, M. and Forssberg, A., Eds., Academic Press, New York, 1961, 436. With permission.)

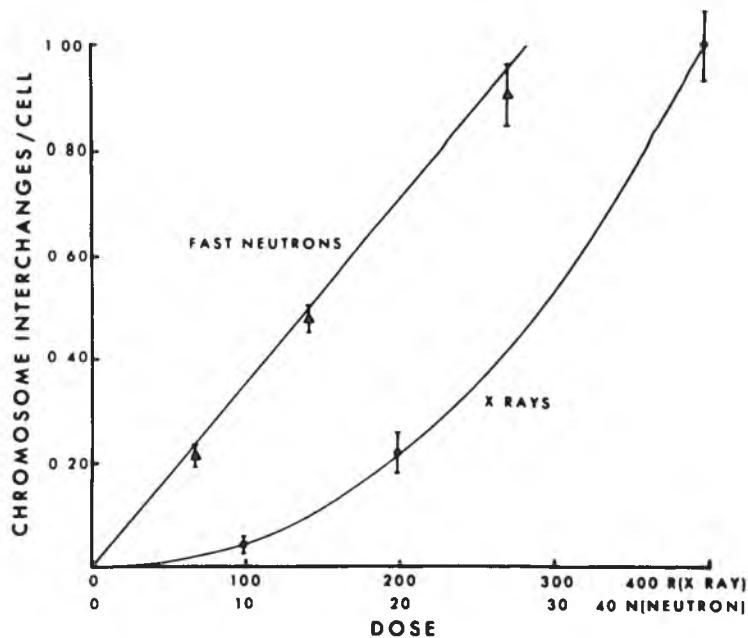


FIGURE 16.3. Frequency of chromosome interchanges vs. dose. 1 N unit = 2.6 R. (From Wolf, S., *Mechanisms in Radiobiology*, Errera, M. and Forssbert, A., Eds., Academic Press, New York, 1961, 440. With permission.)

1. Dominant Single-Gene Disorders:
 - a. Polydactyly (extra fingers and toes)
 - b. Achondroplasia (short-lived dwarfism)
 - c. Huntington's chorea (progressive degeneration of neurons)
 - d. Muscular dystrophy
 - e. Several kinds of anemia
 - f. Retinoblastoma
2. Recessive Mutations on Sex-Linked Chromosomes: This type of recessive mutation expresses in the F_1 generation and occurs exclusively in the male. Examples are hemophilia (failure of the blood to clot), color blindness, and a severe form of muscular dystrophy.

B. REGULAR RECESSIVE MUTATIONS

Recessive mutations do not appear until the two gametes from the male and female, having the same mutation, combine. However, this may not occur for several generations. There may be a good chance that a mutant gene may be eliminated from the population before it has a chance to encounter another like itself.² Examples of regular recessive mutations include Tay-Sachs disease, sickle cell anemia, and cystic fibrosis.

C. CHROMOSOMAL DAMAGE

Diseases associated with chromosomal disease include Down's syndrome (trisomy 21) and embryonic death.

D. MULTIFACTORIAL DISEASES OR IRREGULARLY INHERITED DISEASES

Diseases such as cancer, heart disease, hypertension, and atherosclerosis have genetic and quasigenetic origins, and are included in this group. Table 16.1 describes the spontaneous rate of genetic diseases in humans.

E. MUTATIONS DUE TO CHROMOSOMAL DAMAGE

This type of mutation may be caused by an error in the distribution of the chromosome and by actual breakage of the chromosome.

1. Error in the Distribution of Chromosomes

An error in distribution can cause an uneven distribution of chromosomes in the gamete. Gametes, then, may contain too many or too few chromosomes. For example, Down's syndrome is caused by the presence of an extra #21 chromosome. Generally, gametes having too many or too few chromosomes lead to embryonic death.² Low doses of X-irradiation generally do not cause this type of mutation.²

2. Breakage of Chromosomes

Ionizing radiation often causes this type of chromosomal damage, which can lead to loss, duplication, and rearrangement of genetic materials.

Examples of chromosomal damage that results in loss of genetic materials are (1) terminal deletion, which requires one break, and (2) interstitial deletion, which requires two breaks (Figure 16.4).

1. *Terminal deletion:* If a chromosome has broken into a piece containing a centromere and an acentric piece, the broken fragment may rejoin in the original configuration. However, if the broken piece does not join, this piece will be lost. The daughter nucleus, after mitosis, will always have reduced genetic materials.
2. *Interstitial deletion:* When the chromosome is broken in two different places, the middle piece may be lost, and the terminal broken fragments may rejoin. The daughter nucleus, after mitosis, will again have reduced genetic materials.

The genetic consequences of terminal deletion and interstitial deletion are similar, and they depend upon the size of the chromosome segment lost. Some possibilities are given below.

1. Loss of a large segment may cause dominant lethal mutation.
2. Loss of a medium-sized segment may cause recessive lethal mutation.
3. Loss of a small-sized segment may cause visible mutation.

TABLE 16.1
Estimate of Spontaneous Rate of Genetic Diseases in Humans

Type of Genetic Damage	Number per 1000	Incidence Live Births
Dominant	10	10 ^a
X-linked	0.4	—
Recessive	2.5	2.5
Chromosomal aberration	4.4	6.3
Congenital abnormalities	20–30	60
Other multifactorial	1200 ^b	600 ^a

Data were summarized from BEIR V., 1990.

^a Estimated by U.N. Scientific Committee on the Effects of Atomic Radiation (UNSCEAR).

^b Estimation by BEIR, V. 1200 cases/1000 liveborn does not make any sense in spite of the explanation provided.

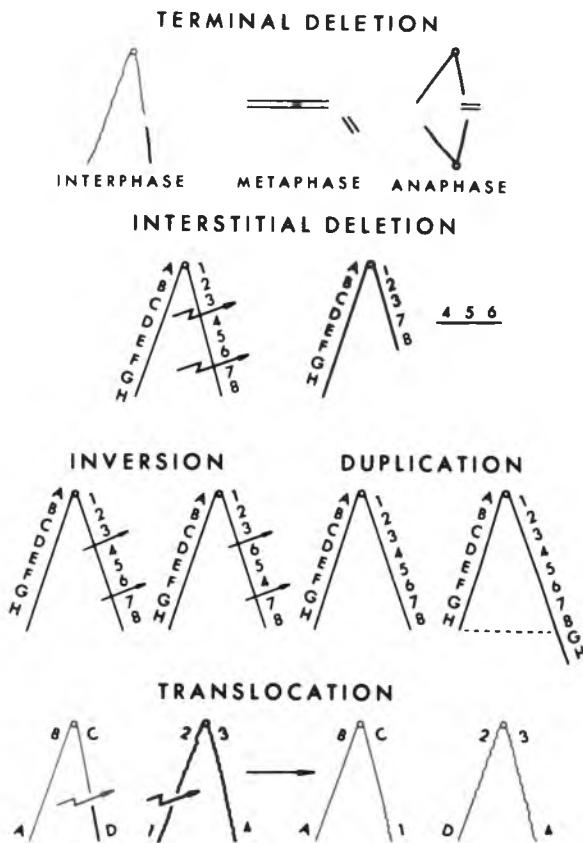


FIGURE 16.4. Schematic representation of various chromosomal aberrations.

Loss of a small segment of chromosomes may have consequences quite similar to, and often operationally indistinguishable from, single-gene mutation.²

a. Duplication

This type of chromosomal damage requires one break. The free piece of the chromosome may be added onto another chromosome, so that the cells, after mitosis, contain a duplication for certain genes (Figure 16.4). Duplications are generally more viable than deficiency. Duplication of a large segment of chromosome may cause dominant lethal mutation.

Examples of chromosomal damage due to rearrangement of broken fragments of chromosomes are (1) translocation, which requires two breaks; (2) inversion, which requires two breaks (Figure 16.4); and (3) dicentric rings, which require two breaks.

b. Translocation

The exchange of segments between two or more chromosomal rearrangements is not harmful as long as the normal gene contents are present. However, the gametes of persons having such "balanced" translocations frequently receive only one of the two parts of the rearrangements; and the zygotes produced by such gametes are genetically unbalanced.² The nature and the extent of abnormality of the embryo depend upon the particular chromosomal regions that are duplicated or deficient; as well as upon the size of the chromosome.² Most of the zygotes having unbalanced genetic materials result in early embryonic death. However, the survivors of such chromosomal damage often have physical abnormalities associated with mental retardation.

c. Inversion

If the two breaks are in the same chromosome, then the interstitial piece — instead of being deleted — is inverted and reinserted into the chromosome. The frequency of inversion is much less than translocation.² The genetic consequence of this type of chromosomal damage is semisterility. However, the extent of the effect depends on the size and location of the chromosomal segment that is inverted.

d. Dicentric Ring

The rearrangement of chromosomes after two breaks can lead to the configuration of a dicentric ring.

These studies show that ionizing radiation can induce deletion, duplication, and rearrangement of chromosomes. The incidence of small deficiency and duplication may be higher than gross rearrangements at lower radiation doses.² Zygotes carrying larger deficiency and duplication may be lost because of high genetic imbalance.

3. Nondisjunction of Chromosomes

The nondisjunction of a chromosome may produce trisomy and monosomy.² Monosomy is generally lethal at a very early embryonic stage. However, when sex chromosomes are involved, X-chromosome monosomy (X/O) leads to Turner's syndrome, characterized by amenorrhea and various morphological abnormalities.² Monosomy can be produced by mechanisms other than nondisjunction of the chromosome; therefore, there is no correlation between the frequency of monosomy and trisomy. Trisomies in humans are produced when sex chromosomes as well as a number of autosomal chromosomes are affected.² Trisomies may result in early death or may cause Down's syndrome, afflicting the bearer throughout life.

Radiation-induced trisomies in humans are not conclusive. Some studies have shown that the irradiation of female gonads before conception enhances the risk of Down's syndrome among children.³⁻⁵ A high frequency of Down's syndrome has been reported among the population of Kerala, India, who are exposed to high background radiation (1.5–3 R/year);⁵ others have failed to confirm the above observation.⁶⁻⁹ Ionizing radiation has been shown to induce trisomy in insects.¹⁰

VI. RADIATION-INDUCED MUTATIONS IN MAMMALS

A. EFFECT OF DOSE RATE IN MALES

The frequency of mutation in mice is dependent on the dose rate.¹¹ Figure 16.5 shows that the irradiation of spermatogonia at a lower dose rate (0.009 R/min) markedly reduces the frequency of mutations in comparison to those irradiated with the same total dose at 90 R/min. A reduction of the dose rate from 0.009 to 0.001 R/min produced no further decrease in mutation frequency. In males, there is no evidence of a threshold dose rate. No dose rate effect on mouse spermatogonia was observed with fission neutrons.

B. EFFECT OF DOSE RATE IN FEMALES

The population of oocytes is fixed at birth and, therefore, provides ideal material for the measurement of the dose rate effect on the frequency of mutations. Sufficient data are available to show that the dose rate effect on mutations in oocytes is quite different from that observed in spermatogonia:

1. The mutation frequency in oocytes at a high dose rate (90 R/min) is greater than that of spermatogonia.

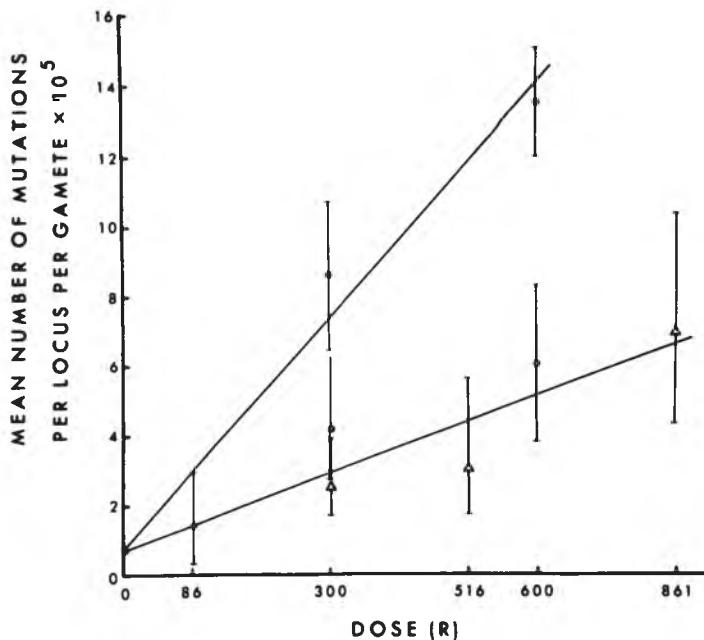


FIGURE 16.5. Specific locus mutation rates, with 90% confidence intervals for various doses and dose rates, in spermatogonia of mice. The lower straight line is fitted to the combined 0.001 R/min (open circles) and 0.009 R/min (open triangles) dose rate data. The upper line is fitted to the 90 R/min (solid circles) points. (From Russell, W.L., *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*, Sobel, F.H., Ed., Macmillan, New York, 1963, 206. With permission.)

2. The mutation frequency in oocytes at a low dose rate (0.009 R/min) is much less than that of spermatogonia.
3. A drop in frequency of mutation from 90 to 0.009 R/min is much greater in oocytes than in spermatogonia.
4. In spermatogonia, there is no significant difference in the frequency of mutation between dose rates of 0.8 and 0.009 R/min; however, in oocytes there is a marked difference.
5. The frequency of mutations at 0.009 R/min in females, even at doses of 400 and 258 R, is similar to those of spontaneous mutations in the male (Table 16.2).

TABLE 16.2
Comparison between Spontaneous Mutation in
Males and Radiation-Induced Mutation at Low
Dose Rate (0.009 R/min) in Females

Sex	Dose rate (R/min)	Dose (R)	Mean Number of Mutations per Locus per Gamete $\times 10^5$
Male	0	0	0.75
Female	0.009	258	0.53
Female	0.009	400	0.77

Data were summarized from Russell, W.L., *Proc. Natl. Acad. Sci. U.S.A.*, 54, 1552, 1965.

TABLE 16.3
Mutation Rate in Male Mice

Type of Radiation	Mutation Rate
X-ray, high dose rate	10^{-5} – 10^{-4} per rad
X-ray, low dose rate	10^{-8} – 10^{-7} per rad
Neutron, high dose rate	RBE of 5–7
Neutron, low dose rate	RBE of 20 or more

There is no reliable estimate for the spontaneous mutation rate in females, but it does not appear to be different from that at 0.009 R/min. A low dose rate of X-ray decreases the frequency of mutation induced by a high dose rate by a factor of 3, whereas a low dose rate of neutron radiation increases the frequency of mutation induced by a high dose rate by a factor of about 4 (Table 16.3).

C. AGE AND DOSE RATE EFFECT

When old mice (6–9 months) or young mice (2–4 months) were X-irradiated, there was no significant difference in the frequency of mutations in the first postirradiation litters of old mice or any postirradiation litters of young mice. However, the second litter of old mice showed a considerably higher mutation rate (Table 16.4). The reasons are unknown. The fact that the mutation frequency in second litters of old females irradiated at 0.8 R/min is similar to that of the first litters of older females irradiated at 90 R/min suggests the possibility that the oocytes involved in the production of these second litters may have reduced capacity for repair of premutational damage. However, the fact that the frequency of mutations in the second litters of old females is also dependent upon the dose rate indicated that some repair does occur.

D. EFFECT OF FRACTIONATION

Figure 16.6 shows that the time intervals between fractionations are crucial for the frequency of mutations. The highest frequency of mutations in spermatogonia is obtained when 1000 R is delivered in two fractions 24 hr apart. The reasons for this phenomenon are unknown. However, in females, a fractionation interval of 24 hr does not influence the frequency of mutations (Table 16.5). When the interval between fractionations was shorter (75 min), an exposure of 400 R would produce a lower frequency than that of a single dose. One

TABLE 16.4
**Effect of Age on Radiation-Induced Mutation
in Female Mice Exposed to 400 R**

Approximate Exposure Rate (R/min)	Mutations per Locus per Gamete $\times 10^5$	
	All Litters of Young and First Litter of Older Females	Second Litter of Older Females
0.009	0.64	3.90
0.8	4.16	18.85
90.0	19.26	—

Data were summarized from Russell, W.L., *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*, Sobel, F.H., Ed., Macmillan, New York, 1963, 205.

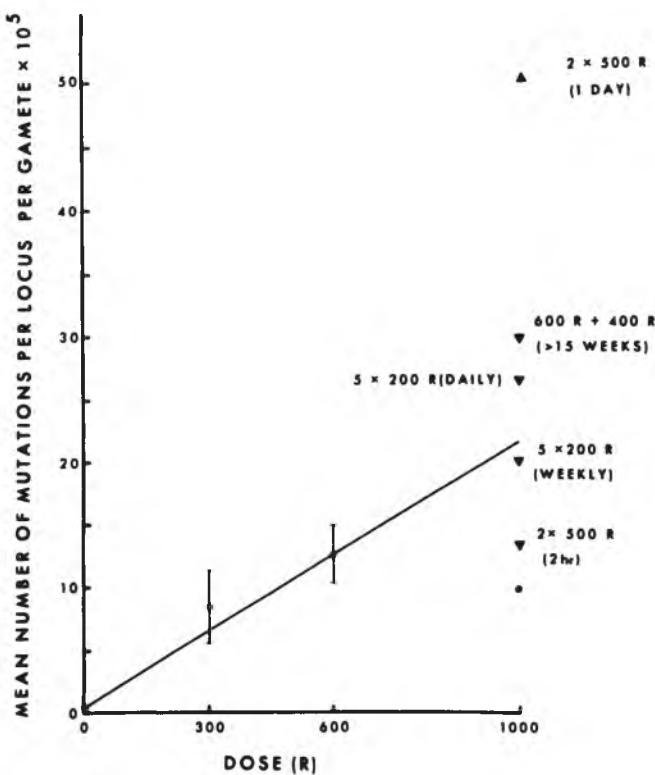


FIGURE 16.6. Mutation rates at specific loci in spermatogonia of mice exposed to fractionated (solid triangles) and single (open circles) dose of acute (90 R/min) X-irradiation. The straight line is fitted to the control and the 300 and 600 R single exposure points. (From Russell, W.L., *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*, Sobel, F.H., Ed., Macmillan, New York, 1963, 214. With permission.)

study¹⁶ has shown that fractionation reduces the number of translocations (Table 16.6), but an increase in the time interval between fractionations did not affect the incidence of mutations.

E. EFFECT OF HIGH LET RADIATION

Table 16.7 shows that the RBE value of neutrons for the production of mutations in spermatogonia is higher when delivered as chronic exposure rather than acute exposure.¹³ Neutron irradiation of mature oocytes shows an RBE value similar to that of spermatogonia.¹²

TABLE 16.5
Mutation Frequency in Female Mice Exposed
to X-Irradiation at 90 R/min

Total Exposure (R)	Mode of Delivery	Mutations per Locus per Gamete $\times 10^5$
400	Single	20.6
400	200 R + 24 hr + 200 R	21.1

Data were summarized from Russell, W.L., *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*, Sobel, F.H., Ed., Macmillan, New York, 1963, 205.

TABLE 16.6
Effect of Fractionation on the Frequency
of Translocation in Male Mice

Total Dose (rads)	Number of Fractionations	% Translocation
600	12 (one/day)	6.09 ± 0.72
600	12 (one/week)	7.09 ± 0.9
630	1	11.5 ± 1.3

Data were summarized from Lyon, M.F., Phillips, R.J.S., and Glenister, P.H., *Mutat. Res.*, 15, 191, 1972.

F. EFFECT OF THE TIME INTERVAL BETWEEN IRRADIATION AND CONCEPTION

The time interval between irradiation and conception is also very important.¹² The frequency of mutation was high when the time interval between irradiation and conception was 7 weeks or less. When radiation was delivered 7 weeks after conception, no mutations were seen (Table 16.8).

G. SPECIES DIFFERENCE

The incidence of dominant lethality induced by 400 R of X-irradiation given to spermatozoa is about 50% higher for rats than for mice.¹⁴

H. DOES RADIATION-INDUCED MUTATION REPAIR?

Repairs of premutational change occur.¹¹ This view is supported by results obtained from microorganisms.¹⁵ At high dose rates, the repair processes in spermatogonia and oocytes are probably damaged. The frequency of mutations appears to be dependent on intermediate steps between the initial radiation damage and completion of the mutation. Therefore, it is possible that a variety of factors, in addition to the dose rate, may change the incidence of radiation-induced mutation by influencing the intermediate steps. The steps involved between the initial radiation damage and the completion of mutations remain to be defined. However, the discovery of factors that modify the frequency of mutations may help in defining these steps.

VII. ESTIMATION OF GENETIC RISK IN HUMANS

The estimation of genetic risk is currently based primarily on rodent data rather than on *Drosophila* genetics. Mutation doubling dose in humans is 100 rem (1 sv). It is based on mouse data for recessive mutation at specific loci.¹⁸ The doubling dose of mutation is calculated as spontaneous mutation rate divided by the rate of induction of new mutations per rem.

TABLE 16.7
RBE Neutron for the Production of Mutation

Cell Type	RBE Value		
	Acute Exposure	Chronic Exposure	Reference
Spermatogonia	4	20	13
Mature oocytes	5	Not known	12

TABLE 16.8
Mutation Frequency at Two Intervals After
Irradiation in Female Mice Exposed to a
Dose of 50 R at 90 R/min X-Irradiation

Interval Between Irradiation and Conception	Number of Mutations at 7 Loci per 105 Offspring
Up to 7 weeks	20.15
More than 7 weeks	0

The data were summarized from Russell, W.L., *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*, Sobel, F.H., Ed., Macmillan, New York, 1963, 205.

Relative mutation risk is 1/100. Prevalence (P) of autosomal and X-linked mutations is 10,000 cases per 10^6 liveborn children. Risk at equilibrium per rem = $P \times$ relative mutation risk = 100 cases/ 10^6 liveborn children. Risk to F_1 generation = 6.6 cases/ 10^6 liveborn children. Continuous exposure of a population to the background radiation of 3 rem/30-year generation would account for only 1–6% of the spontaneous mutation rate.² Current incidence of human genetic disorders resulting from causes other than radiation = 107,000 cases per 10^6 offspring. Table 16.9 describes the effect of irradiation on genetic damage in humans.

All radiation exposures are of genetic importance. Genetic significant dose (GSD) is a measure of population gonadal exposures and equals 80 mrem/year/person exposures. The sources of GSD per year are as follows:

Diagnostics	20 mrem
Therapeutics	3 mrem
Occupational	1 mrem
Fallout	1.5 mrem
Man-made sources	30–40 mrem

The total genetic effect in the population will be the same whether the entire population is exposed to 80 mrem/year or each member of the population receives 80 mrem/year.

TABLE 16.9
Estimated Genetic Effects/rem per Generation

Type of Disorder	Current Incidence per 10^6 Liveborn	First Generation	Additional Cases/ 10^6 Liveborn/rem at Equilibrium*
Autosomal			
Dominant	10,000	6–35	100
X-linked	400	< 1	< 5
Recessive	4,400	< 6	< 1
Congenital	20,000	10	10–100
Anomalies	30,000		

Data were summarized from BEIR, V, 1980. The risk of other disorders of complex etiology such as cancer, heart diseases, and other was not estimated.

* Equilibrium = perhaps 10 generations later.

VIII. SUMMARY AND COMMENTS

The frequency of mutations in mice is dependent upon the dose rate. When the dose rate is reduced, the incidence of mutations decreases. The dose rate effect on mutations in female mice is different from that observed in males. The mutation frequency in oocytes, at a high dose rate, is greater than that of spermatogonia; but at a lower dose rate, the frequency in oocytes is much less than that of spermatogonia. A drop in the frequency of mutations from 90 to 0.009 R/min is much greater in oocytes than in spermatogonia. In spermatogonia, there is no significant difference in the frequency of mutations between dose rates of 0.8 and 0.009 R/min; however, in oocytes there is a marked difference. Age is also an important factor in the dose rate effect. The time interval between fractionations is quite critical for the production of mutations in the male. The RBE of an acute neutron dose for the production of mutations is about 5 to 6 in both spermatogonia and oocytes. The time interval between irradiation and conception is very important for the production of mutations.

The fact that the frequency of mutations decreases when the dose rate is reduced indicates that repair of premutational change occurs. Since mutant genes are quite stable, a repair of a completed mutation frequency may help in defining the steps involved between initial radiation damage and completion of mutation.

Continuous exposure of a population to background radiation of 3 rem per generation of 30 years would account for only 1–6% of the spontaneous mutation rate. Therefore, a small increase in background radiation would incur a relatively small additional risk in the rate of spontaneous mutations. The parental exposure of 1 rem before conception for several generations would increase the mutation rate by about 60 to 1100 per 10^6 offspring. On the other hand, average parental exposure of 1 rem before conception for a period of one generation can be expected to produce an additional 5–65 mutation cases per 10^6 offspring. Risk estimate among irradiated males is 10–20 cases per 10^6 liveborn children/rem, whereas among irradiated females it is 0–9 cases per 10^6 liveborn children/rem. The lower estimate in females is due to the fact that mouse data reveal that females may be less sensitive to induced mutation than males.¹⁸

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RADIATION CARCINOGENESIS: TISSUE CULTURE MODEL

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I. INTRODUCTION

Radiation-induced neoplasms cannot be distinguished from those that occur spontaneously or that are induced by chemicals. The structural and functional changes in carcinogenesis may be similar irrespective of causative agents. Cancer cells result from accumulation of multiple genetic changes in normal dividing cells, which occur over a long period of time. The latent period (time interval between exposure to carcinogens and detection of cancer) of a neoplasm depends upon the experimental models. Three models are used to study radiation carcinogenesis: the tissue culture model, the animal model, and the human model. The latent period of cancer is about a few weeks in the tissue culture model, about a few months in the animal model, and several years in the human model. Each model will be discussed in a separate chapter.

A. CARCINOGENESIS

Experiments *in vivo* and *in vitro* utilizing chemicals and radiation identified three distinct steps in carcinogenesis: (1) initiation, (2) promotion, and (3) progression.

1. *Initiation:* Initiating events^{1,2} in chromosomes (aberrations) or in DNA (mutations) following radiation exposures may occur. The nature of these initiating events is unknown. If the radiation dose is sufficient, one or two of these cells may become cancerous. If the radiation dose is low, the initiated cells may require exposure to additional radiation or chemical carcinogens to become cancer cells. Examples of tumor initiators are ionizing radiation, ultraviolet radiation, nitrosamine, and benzopyrene.
2. *Promotion:* Promoting events^{1,2} in cells exposed to low doses of tumor initiators are essential to convert them to cancer cells. The nature of these promoting events is not known. Generally, the tumor promoters even at high doses are not sufficient to convert normal (uninitiated) cells to cancer cells. Tumor promoters can enhance the incidence of radiation-induced cancer and reduce its latent period. Examples of tumor promoters are phorbol ester, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), estrogen, and excessive fat.

3. **Progression:** Increased genetic instability due to additional chromosomal aberrations or mutations in one or two cancer cells can make them more aggressive, resulting in rapid spread of cancer to distant organs. The process of tumor cell invasion is referred to as metastasis. The nature of the changes that make cancer cells more aggressive is not known. The three-step model of carcinogenesis was very useful in identifying agents that are tumor initiators, tumor promoters, and antitumor promoters, but it failed to identify molecular steps that are necessary for initiating carcinogenesis.

Based on numerous cellular and molecular biology studies, the following hypotheses of cancer have been proposed:

1. Activation of protooncogenes.^{3,4}
2. Loss of antioncogenes.⁵
3. Infection with certain viruses.⁶
4. Substitution of normal promoters of protooncogenes with strong promoters of viruses.⁷
5. Chromosomal aberrations.^{8b}

A recent critical analysis of these hypotheses has concluded that — except for the chromosomal aberrations hypothesis — none of the other hypotheses can be considered plausible, because only chromosomal damage is consistently observed in tumors with or without mutated cellular oncogenes and with or without latent viruses.^{8a} The oncogene gene hypothesis could not explain the following:

1. Cellular oncogenes (*c-myc* and *c-ras*) that, unlike viral oncogenes, possess weak promoting elements, failed to transform human epithelial or fibroblast; although viral oncogenes that contain strong tumor promoting elements do.
2. The transforming ability of retroviral oncogenes depends primarily on high transcriptional activity which, in turn, depends upon an active promoting region. An abnormality in oncogene expression occurs only in up to 50% of any given tumor.
3. The antioncogene *rb* gene failed to suppress tumorigenicity upon transfection of cancer cells. Therefore, the initial oncogene hypothesis was modified. This modified hypothesis proposes that cancer cells are the result of an accumulation of multiple abnormal cellular oncogenes in normal cells.

Radiation-induced neoplastic transformation *in vitro* and *in vivo* is also associated with alterations in the expression of certain oncogenes.⁴⁶⁻⁵¹ Activation of *k-ras*⁴⁶ and the presence of *k-ras* and *N-ras* mutations⁴⁷ have been demonstrated in some of the X-ray-induced lymphoma in mice. *K-ras* mutation and increased expression of *c-myc* were also present in some of the rat skin tumor induced by radiation.⁴⁸ Neutron-induced *k-ras* mutation is different from that produced by γ -radiation.^{49,50} Although the *ras* gene abnormality was detected in some cases of radiation-induced tumors, it is less frequent compared with that of certain chemical carcinogens. The frequency of abnormality in oncogene expression is seldom over 50% either in human tumors or in chemical-induced animal tumors. Therefore, the involvement of *ras* or other cellular oncogenes in radiation-induced cancer is incidental. It has been reported that an unidentified oncogene may be involved in α particle-induced transformation *in vitro*.⁵

We have proposed a four-stage hypothesis which is described below:

1. **Chromosomal damage in normal dividing cells:** Chromosomal damage in normal dividing cells occur after radiation exposure. Such cells divide, differentiate, and die like undamaged normal cells.

2. *Defect in differentiation genes:* One or two cells from the above cell population develop a defect in differentiation genes, which prevents them from a normal pattern of differentiation and death. These cells then continue to divide, become hyperplastic, and develop into adenoma. Examples are adenoma of the parotid glands, polyps of the colon, and cyst of the breast (mastitis). The differentiating genes have not been identified.
3. *Gene defect in hyperplastic cells:* One or two hyperplastic cells in any adenoma can accumulate additional gene defects due to mutations or chromosomal damage, which can make them cancerous. Scattered foci of cancer cells in the colon polyp is an example. These cancer cells can metastasize to distant organs. The genes responsible for converting hyperplastic to cancer cells have not been identified.
4. *Gene defect in cancer cells:* One or two cancer cells can accumulate additional gene defects due to mutations or chromosomal damage, which can make them more aggressive. No such genes have been identified, although several claims of aggressive cancer genes have been reported, e.g., *N-myc* in neuroblastoma and *erb* in breast cancer. Unfortunately, only a small percentage of aggressive tumors show such defects.

Monolayer cultures of normal embryonic cells have been used to establish dose–effect relationships for the induction of cancer by radiation. Generally, the presence of transformed cells in a culture after irradiation has been considered an index of carcinogenic change. There is no doubt that one can study the mechanisms of radiation-induced carcinogenesis in cell culture models with great ease and precision; however, the dose–effect relationship cannot be extrapolated to whole organisms. Nevertheless, one can develop new principles and concepts that may be applicable to humans in a qualitative sense.

II. TYPE OF CELLS AND DOSE–RESPONSE CURVE

Syrian hamster embryo cells (C3H 10T1/2) and the A31-11 line of mouse BALB/3T3 have been extensively used for radiation-induced transformation; however, radiation-induced transformation in human skin cells has also been observed. The transformed cells grow and form colonies in the medium. They can be identified by their growth pattern (they grow in clumps). When they are injected into syngeneic mice, they may or may not form tumors. Only those transformed cells that form tumors in athymic mice are considered cancerous. Those that do not form tumors should be called immortal. It has been reported¹¹ that the dose–response curve for the induction of transformation after a single dose is very complex. The following three types of dose–responses have been observed:¹¹

1. Above 100 rads: the transformation frequency may exhibit a quadratic dependence on doses.
2. Between 30 and 100 rads: the transformation frequency may not vary with dose.
3. Below 30 rads: the transformation frequency may be directly proportional to dose.

These results show that a linear extrapolation from results at high doses cannot provide an accurate estimation of the transformation incidence at low doses.¹¹

Another type of dose-response curve (Figure 17.1) shows that transformation per cell exposed increases up to 400 rads (4 Gy), after which it decreases (Figure 17.1). The latter phenomenon is due to the fact that cell survival also decreases significantly after 4 Gy, and fewer cells remained to be transformed.

It has been reported that fresh explants of hamster embryo cells are ten times more sensitive to X-ray-induced transformation than established line C3H 10T1/2 cells.¹ Doses as low as 1 rad of X-rays and 0.1 rad of neutrons can produce transformation in fresh explants of hamster

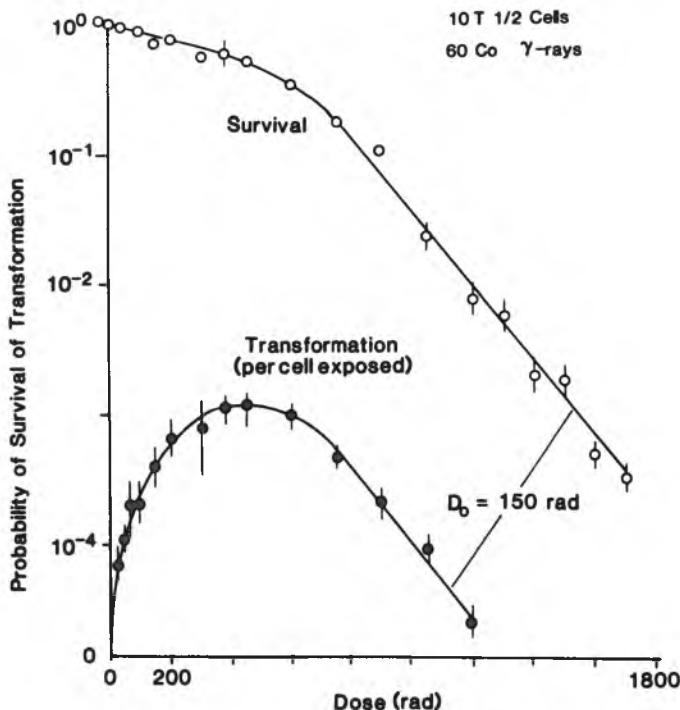


FIGURE 17.1. Probability of survival (top) and transformation per irradiated cell (bottom) as a function of dose.²⁵

embryo cells.¹² It has been reported¹² that radiation-induced transformation can be produced in embryos of Syrian hamsters irradiated (300 rads) *in utero* and assayed *in vitro*. However, the frequency of transformation is at least ten-fold lower than that obtained when the cells are irradiated *in vitro*. This frequency (0.07–0.1%) of transformation is close to the incidence of radiation-induced tumors in animals.¹²

More recent studies suggest that human epidermal keratinocytes immortalized by transfection with adenovirus type 12 and SV40 can be transformed to a malignant state by X-irradiation with a dose of 4–8 Gy.¹⁸ Several other types of rodent and human cells have been immortalized by inserting exogenous genes. These immortalized lines can be very useful in the study of the mechanisms of carcinogens.

Radiation-induced transformation of mammalian cells *in vitro* provides a useful model to study the mechanisms of carcinogenesis. It also provides a sensitive system to study modifications of the carcinogenetic processes. Indeed, several elegant studies on the modifications of radiation-induced carcinogenic processes have been performed.

III. MODIFICATION OF RADIATION-INDUCED TRANSFORMATION

Studies on the modification of the radiation-induced transformation are important, because they may provide new insights into the mechanisms of carcinogenesis. Table 17.1 lists enhancers and Table 17.2 lists protectors of radiation-induced transformations *in vitro*.

A. ENHANCERS OF RADIATION-INDUCED TRANSFORMATION

1. Effect of Fractionation

The effect of fractionated doses on the frequency of transformation also depends upon the total dose.³ At doses of about 150 rads, the fractionation of dose reduces the transformation

TABLE 17.1
Enhancers of Radiation-Induced
Transformation *In Vitro*

Agents	
Fractionation of dose	High-LET radiation
UV radiation	Chemical carcinogen
Viruses	Phorbol ester
Estrogen	Insulin
Asbestos fiber	Iron
Hyperthermia (43°–45°C)	Ozone

frequency in comparison to that produced by a single dose. However, at between 30 and 100 rads the fractionation of dose enhances the frequency of transformation (Figure 17.2). Furthermore, at 100 rads the division of the dose into more fractions leads to a progressive increase in transformation frequency.

2. Effect of High-LET

High-LET radiation (helium-3 ions) is more effective in causing transformation in surviving rodent fibroblasts than γ -radiation (Figure 17.3). On the other hand, the transformation per cell at risk decreases in proportion to decreases in cell survival following irradiation with both high- and low-LET radiation. The combination of high-LET and asbestos fibers increases the transformation frequency in C3H 10T1/2 mouse cells by more than additive.¹⁷

3. Effect of Tumor Promoter

The active principal in croton oil is a class of compounds called the phorbol esters. Among these, 12-O-tetradecanoyl phorbol-13-acetate (TPA) is one of the most active tumor promoters for chemically initiated carcinogenesis. It has been observed¹³ that TPA also markedly enhances the frequency of X-ray-induced transformation (Figure 17.4). It is interesting to note that TPA is particularly effective at low doses. For example, a dose of 25 rads does not produce detectable levels of transformation; however, when this dose is followed by TPA treatment, the transformation frequency increases to 5×10^{-4} . This frequency of transformation is generally induced by 200–300 rads of X-rays alone.¹³ TPA appears to be most effective during the proliferative phase of growth. It has been reported¹⁵ that the irradiated cells retain the ability to respond to TPA even after many generation times. This observation is consistent

TABLE 17.2
Protectors of Transformation
In Vitro^a

Agents	Cell type
Protease inhibitors	C3H 10T1/2
Vitamin E	C3H 10T1/2
Ascorbic acid	C3H 10T1/2
β -carotene	C3H 10T1/2
Retinoids	C3H 10T1/2
Cortisol	C3H 10T1/2
Sphingolipid	C3H 10T1/2

^a C3H 10T1/2, a mouse embryo fibroblast; sphingolipid, a protein kinase C inhibitor; vitamin E, α -tocopheryl succinate.

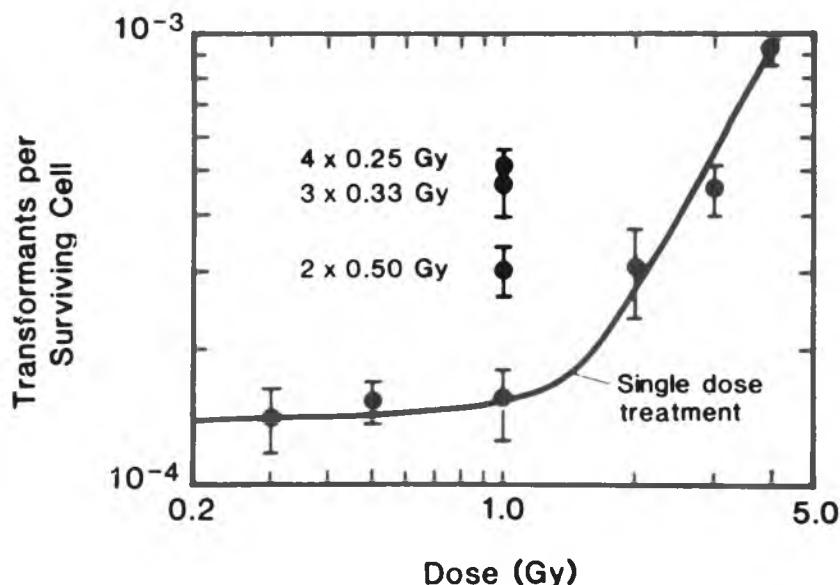


FIGURE 17.2. The transformation incidence after irradiation of cells with a dose of 3000-kVp X-rays delivered in a single acute exposure or divided into three or four equal fractions spread over 5 hr. (From Hall, E.J., *Radiat. Res.*, 87, 217, 1981. With permission.)

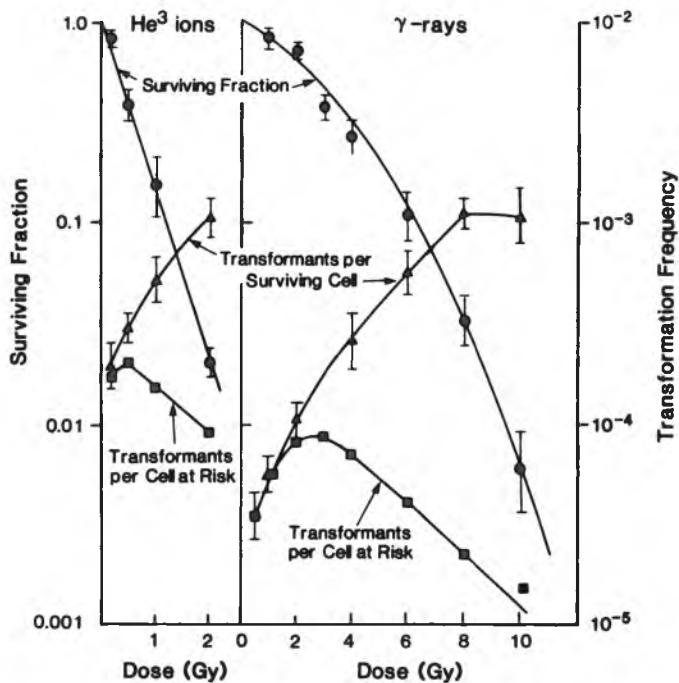


FIGURE 17.3. Cell survival curves and dose-response relationships for oncogenic transformation for C3H 10T1/2 cells irradiated with either γ -rays or high-LET helium-3 ions. Transformation frequencies are expressed in two ways: per surviving cell, and per cell initially at risk.²⁴

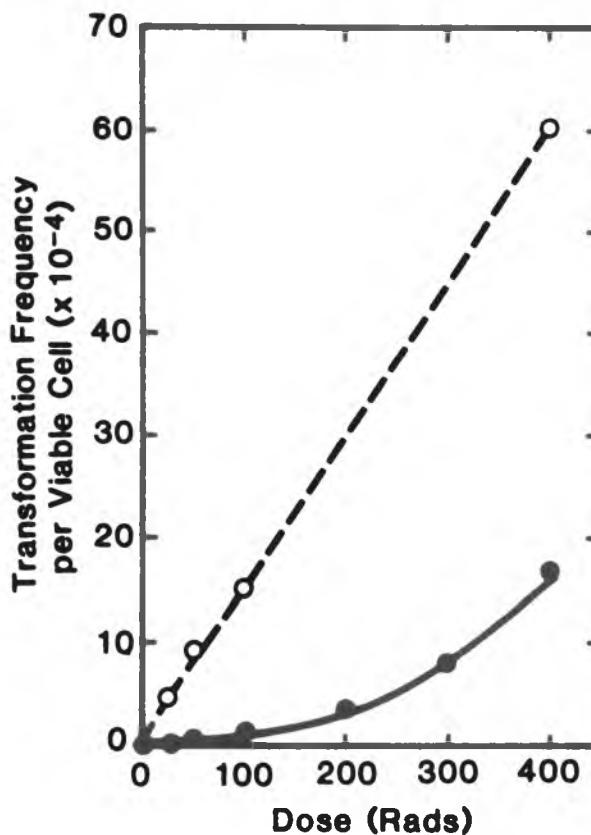


FIGURE 17.4. Influence of postirradiation incubation with tumor promoter TPA on X-ray transformation of mouse C3H 10T1/2 cells. X-irradiation only (O); TPA (0.1 μ g/ml) added for entire expression period (●). (From Little, J.B., *Radiat. Res.*, 87, 244, 1981. With permission.)

with the results found in mouse skin, where applications of croton oil many months after carcinogen exposure still leads to a high incidence of tumors.¹⁶

4. Effect of Metals

Certain heavy metals appear to modify the incidence of chemically induced tumors in animals. For example, methylmercuric chloride (CH_3HgCl), in combination with sodium nitrite plus ethylurea, produced a higher incidence of schwannomas in the progeny of the treated pregnant rat — in comparison to that produced by sodium nitrite plus ethylurea.³⁴ Ependymomas were found only in the progeny of pregnant rats that received CH_3HgCl , sodium nitrite, and ethylurea. Lead and chromium are weak carcinogens; but when lead chromate was injected i.m., 64% of the mice developed malignant tumors at the injection site.³⁵ The interaction of radiation with heavy metals is unknown. Because several heavy metals are present in the environment, it would be important to know whether radiation-induced transformation frequency can be modified by heavy metals. An increase in the iron contents of cells and tissue may increase the risk of radiation-induced cancer.³⁶ This is due to the fact that iron may increase the level of oxygen radicals. A human epidemiological study has revealed that increased iron stored in the body enhances cancer risk.³⁷

5. Effect of Chemical and Viral Carcinogens

Using Syrian hamster cells in culture, it has recently been reported that X-irradiation enhanced the chemically induced transformation rate by a factor of 9. It is interesting to note

that X-irradiation by itself does not induce transformation; however, it potentiated the effect of benzo[*a*]pyrene.³⁸ X-irradiation also increased virus-induced neoplastic transformation.³⁹⁻⁴¹ In addition, X-irradiation enhanced the UV-induced transformation *in vitro* by a factor of 12. At a UV irradiation of about 60 erg/mm, there was 8% transformation in fetal hamster cells in culture.⁴² Unlike X-irradiation, chemical carcinogens such as benzo[*a*]pyrene and *N*-acetoxyfluorenyl acetamide do not enhance the rate of UV-induced neoplastic transformation *in vitro*.²² The mechanisms of X-ray-induced enhancement of transformation induced by chemicals and UV irradiation are unknown. Ozone, a major chemical oxidant in the atmosphere by itself, induces neoplastic transformation in hamster embryo cells and mouse fibroblasts.⁴³ It also enhances radiation-induced transformation *in vitro*.

6. Effect of Hyperthermia

Hyperthermia enhances the frequency of radiation-induced transformation *in vitro*.^{44,45} When C3H 10T1/2 mouse embryo cells were exposed to heat (45°C for 15 min or 43°C for 60 min), no transformation was observed; however, when the heat treatment was preceded by exposure to 200 rads of X-rays, the frequency of X-ray-induced transformation increased four- to five-fold.²³

In vitro transformation of mammalian cells by various types of carcinogens provides an extremely useful model to study the mechanisms of transformation in a well-defined chemical environment. In addition, the modification of the rate of transformation by exogenous agents provides new opportunities to assess the hazards and prevention of carcinogenesis by agents commonly present in the environment or in the body. It should be emphasized that the *in vitro* results cannot readily be applied to *in vivo* conditions where additional factors exist which might influence the process of carcinogenesis.

7. Thyroid Hormone and Estrogen

It has been reported¹² that X-ray-induced transformation was dramatically inhibited when the cells (C3H 10T1/2) were maintained in the absence of thyroid hormone. However, the expected frequency of radiation-induced transformation was restored when thyroid hormone (triiodothyroxine, T₃) was added. The critical period of exposure to hormones was a 12-hr period before X-irradiation. These studies show that the thyroid hormone is necessary for the induction and/or potentiation of radiation-induced transformation *in vitro*. This study is very interesting in view of the fact that altered thyroid status modifies the growth and metastatic potential of implanted tumor³⁰ and the survival rate of animals bearing tumors.³¹ Estrogen also enhances the frequency of radiation-induced transformation of mammalian cells in culture.³²

B. PROTECTORS OF RADIATION-INDUCED TRANSFORMATION

1. Effect of Protease Inhibitors

Protease inhibitors dramatically suppress the radiation-induced transformation of cells in culture.¹⁰ A protease inhibitor, such as antipain, by itself does not induce transformations; however, these inhibitors markedly reduce the frequency of transformation. They completely reduce the transformation induced by 400 rads + TPA. A soybean trypsin inhibitor has produced similar results (Figure 17.5).¹⁹ Other protease inhibitors from the potato also reduced the radiation-induced transformation frequency by a factor of 2- to 3-fold.²⁰

2. Hormone

A concentration of 10⁻⁷ M cortisol suppresses radiation-induced transformation *in vitro*.³³

3. Effect of Vitamins

Animal and human epidemiological studies have suggested that β-carotene and vitamins A, C, and E may be important in reducing the incidence of chemical-induced tumors or of

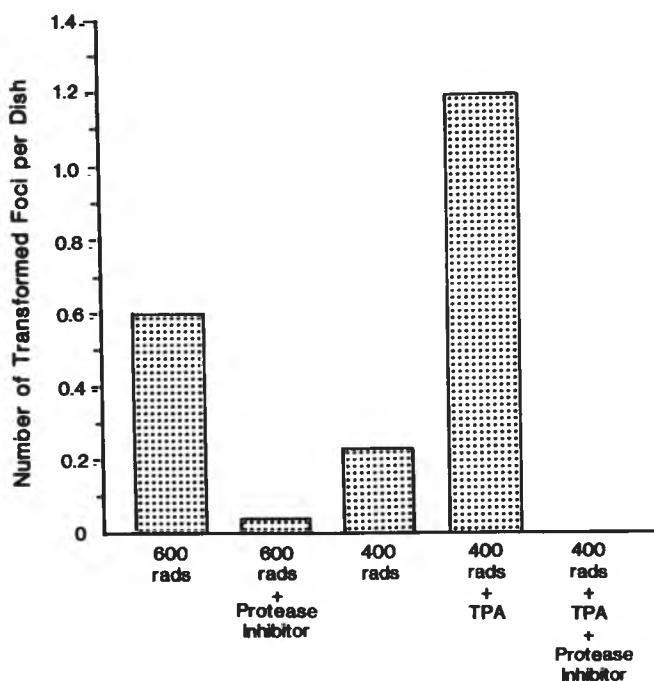


FIGURE 17.5 Suppressive effect of a protease inhibitor (antipain) on radiation transformation *in vitro*, both with and without promotion by TPA.

spontaneously occurring tumors. A vitamin A analog,²¹ α -tocopheryl succinate (α -TS; a most active form of vitamin E²²) and selenium markedly reduced radiation-induced transformation *in vitro*²³ (Figure 17.6). α -TS in combination with selenium is more effective in reducing the level of radiation-induced transformation in rodent fibroblasts *in vitro* than the individual agents (Table 17.3). Vitamin C (0.1–20 μ g/ml), when given after irradiation for the first week, decreases transformation frequency in C3H 10T1/2 cells *in vitro*.²⁶

TABLE 17.3
Effect of α -Tocopheryl Succinate (α -TS) and
Selenium on Radiation-Induced Transformation
of C3H 10T1/2 Cells *In Vitro*^a

Treatments	Transformation Frequency $\times 10^{-4}$
Control	0
Sodium selenite (2.5 μ M)	0
α -tocopheryl succinate	0
400 rads, single dose	9.8 ± 1.2
400 rads + selenium selenite	1.8 ± 0.8
400 rads + α -TS	1.7 ± 0.4
400 rads + α -TS + sodium-selenite	0.9 ± 0.09

^a Sodium selenite and α -TS were given 24 hr prior to irradiation and removed 72 hr later. From Borek et al., *Proc. Natl. Acad. Sci. U.S.A.*, 83, 1490, 1986. With permission. Vitamin E has been replaced by α -TS to emphasize that only this form of vitamin E was active.

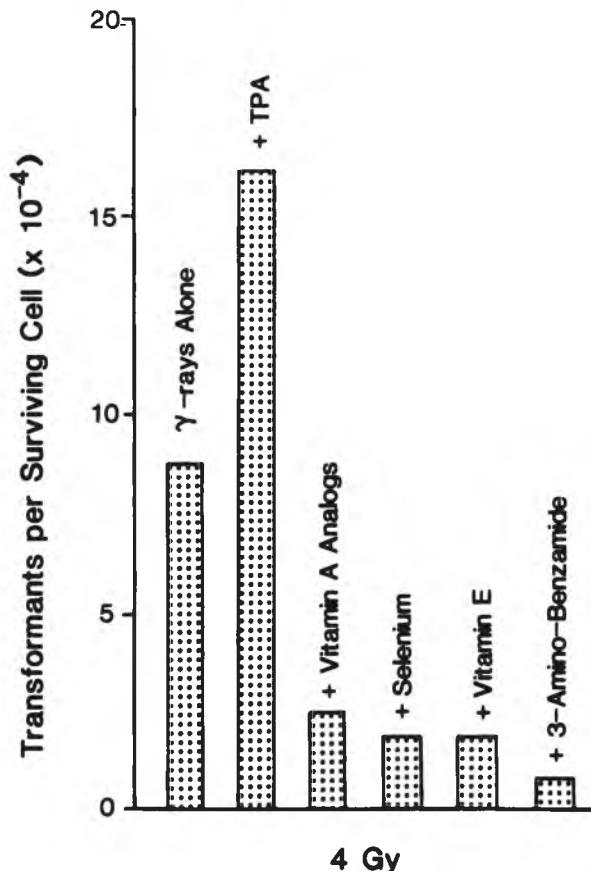


FIGURE 17.6. Effects of vitamin A analogues, selenium, vitamin E, 3-aminobenzamide, and TPA, at 4 Gy and T_3 (thyroid hormone) at 3 Gy on radiation-induced transformation.²⁴

4. Pharmacological Agents

Sphingolipids (inhibitors of protein kinase C) suppressed radiation-induced transformation *in vitro*.²⁷ This suggests that activation of protein kinase C (PKC) may be involved in radiation-induced carcinogenesis *in vitro*. Furthermore, TPA, as expected, enhanced the level of radiation-induced transformation in rodent fibroblasts; however, sphingolipids decrease it by about four-fold.²⁷ Dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), and dimethyl acetamide (DMA), at a concentration of 0.01%, reduced radiation-induced transformation and phorbol ester-induced enhancement of transformation in rodent fibroblasts in culture three-fold.²⁸

5. Effect of Cell Density and Cell Division

There are several factors that influence the transformation of the A31-11 line of mouse BALB/3T3 cells.¹³ For example, the increase in initial cell density decreases the frequency of transformation by several fold. In addition, holding the irradiated cells into a confluent phase of growth for a longer time (greater than 48 hr) reduces the frequency of radiation-induced transformations. This shows that one round of cell division within 24–48 hr of X-ray exposure is required for the transformation.^{13,14}

IV. SUMMARY AND COMMENTS

Cancer cells result from an accumulation of multiple genetic changes which have not yet been identified. Results show that activation of cellular oncogenes is not sufficient for

transformation. The success of radiation-induced transformation in the cell culture model provides an opportunity to study the mechanisms of radiation-induced carcinogenesis. In addition, it is very useful for developing newer concepts regarding the dose-effect relationship. Several agents that increase or decrease the frequency of radiation-induced transformation have been identified. The most important cancer-protective agents are β -carotene, vitamins A, E, and C, and mineral selenium. Cancer-causing and cancer-protective agents may be used as tools to understand the mechanisms of radiation-induced transformation in mammalian cells. It should be emphasized that the results obtained from the tissue culture model cannot be extrapolated to *in vivo* condition. There are several carcinogens, cocarcinogens, tumor promoters, and antitumor promoters in the body that may influence the expression of malignancy after the initiating events of carcinogenesis have started in the cells. In addition, the immune surveillance mechanisms may kill newly transformed cells. Nevertheless, *in vitro* systems are sensitive, cost-effective, and less time-consuming for the study of carcinogenesis.

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Chapter 18

RADIATION CARCINOGENESIS: ANIMAL MODEL

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I. INTRODUCTION

The frequency of radiation-induced cancer has been extensively studied in animals. However, the dose-response curves markedly vary from one tumor type to another.¹ This suggests that other environmental factors, such as cocarcinogens, tumor promoters, and antitumor promoters, may influence the processes of radiation-induced carcinogenesis, thereby shaping the dose-response curve. The factors that influence carcinogenesis include (1) age, (2) sex, (3) genetic constitution, (4) capacity to repair DNA damage, (5) carcinogen metabolism, (6) immunological status, and (7) dietary factors.

A. LEUKEMIA

Several studies show that the incidence of thymic leukemia in RFM mice is dependent upon the dose rate: the higher the dose rate, the greater is the frequency.^{2,3} The incidence of myeloid leukemia in RF male mice is shown in Figure 18.1.⁴ In mice, the smallest dose that causes leukemia is below 200 R. The susceptibility of mice to the induction of leukemia irradiation depends upon sex and strain. In most strains of mice, spontaneous leukemias are thymic in origin. Operative removal of the thymus, or its involution by cortisone, diminishes the incidence of spontaneous leukemias or their induction by irradiation. Removal of the adrenals

increases susceptibility to the induction of leukemia by X-irradiation. Continuous external gamma irradiation throughout life at the rate of 0.062 rad/day failed to induce leukemia in A/Jax mice, but did induce it in 55% of Rap mice.⁵ This may be due to a difference in the genetic susceptibility of the mice.

Although C57BL mice rarely develop spontaneous lymphosarcoma or lymphatic leukemias, they are highly responsive to the leukemogenic action of X-rays. Leukemia induced in C57BL mice by radiation can be transmitted by a cell-free extract. The agent responsible for such transmission is a virus, referred to as a radiation leukemia virus. It has been concluded that mice harbor latent leukemogenic viruses, and that whole-body irradiation (300 R) causes activation and/or release of this virus, or makes the cellular environment compatible for virus replication and, thereby, transforms normal cells into malignant cells.⁶ The leukemia (mostly lymphoma of bone marrow origin) occurred in CBA mice after an i.p. injection of radiostrontium. The occurrence of lymphoma in the bone marrow was inversely related to dose, because the frequency was higher at a lower dose. The mean latency time for all types of lymphomas was 238 ± 5.8 days. No relation between dose and latency time appears to exist for lymphoma.⁷

B. LUNG CANCER

Induction of lung tumor in mice requires a high dose of radiation. Chronic gamma irradiation giving 8 R/day (8 hr), for a total of 2400 R over a period of 10 months, produced a 50% increase in lung cancer. An exposure of 3000 or 4000 R of X-irradiation applied externally to the chest caused 43% of bronchial carcinoma in rats, but only 2% in hamsters.⁸ The inhalation of radioactive particulate also causes lung cancer — 50 μ Ci of ^{144}Ce (2400 rads) causes lung cancer in rats within 78 days.

The effect of the suppression of cell-mediated immunity in radiation-induced lung cancer has been evaluated by removing the thymus prior to irradiation.⁹ Plutonium dioxide (PuO_2) significantly increases (52%) the incidence of pulmonary tumors in control and thymectomized rats (54%); however, the extrapulmonary tumor was four-fold higher in the latter group (17%).

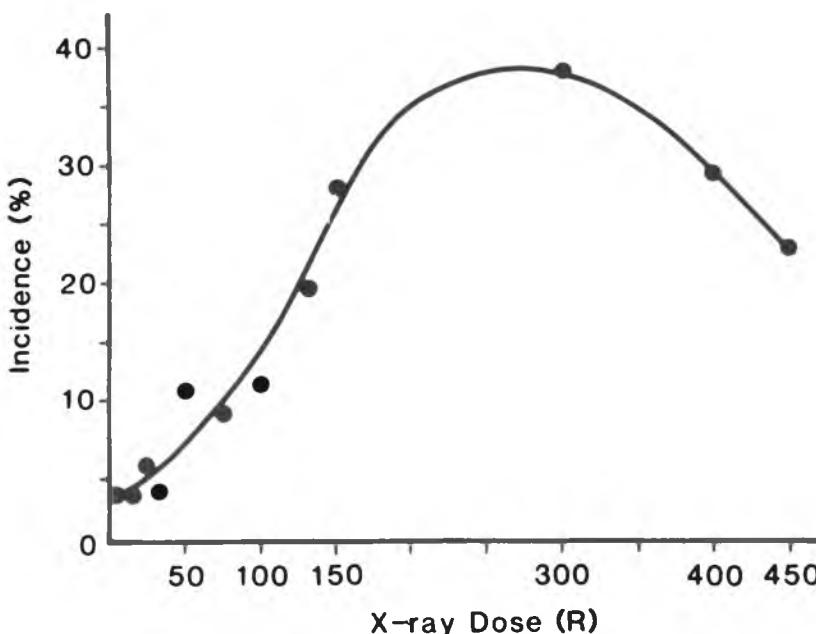


FIGURE 18.1. Incidence of myeloid leukemia in RF male mice exposed to whole-body X-irradiation. (From Upton, A.C., *Cancer Res.*, 21, 717, 1961. With permission.)

than that observed in the former group (4%). It has also been observed⁹ that, in thymectomized animals, the size and invasiveness of the tumor and the frequency of regional metastasis are much higher in comparison to control groups. Thus, it has been concluded⁹ that the immune suppression should not affect the frequency of PuO₂-induced lung cancer, but it may affect the severity of progression of this particular neoplasm.

The effect of secondary factors on ²¹⁰Po-induced lung cancer in the hamster was examined.¹⁰ Administration of 0.1 µCi of carrier-free ²¹⁰Po suspended in 0.2 ml of isotonic saline produces a very low incidence (1–2%) of lung cancer in animals. However, when the animals received 14 weekly tracheal instillations of 0.2 ml isotonic saline, 36% of the animals developed tumors. It was noted that the saline instillations in hamsters induce a transient wave of cell division away from the target epithelial cells of the terminal bronchi. This suggests that saline-induced proliferation of cells acts as a promoter of radiation-induced pulmonary carcinogenesis. The incidence of lung cancer appears to be influenced by dose rate. The incidence of tumor at a dose rate of 8.3 rads/day was less than that observed at a dose rate of 45 rads/min within the dose range of 0–200 rads.

C. BONE TUMOR

Radium (²²⁶Ra) induces bone sarcoma as well as carcinoma. The threshold is about 0.5 µg of radium.

The incidence of sarcoma increases markedly in both humans and beagles after the administration of ²³⁹Pu and ²²⁶Ra.¹² The incidence depends upon the particular model used. Risk estimates of having sarcoma on the basis of linear models is 5200/10⁶ per beagle per rad; whereas on the basis of another model, this value is only 21–22/10⁶ per beagle per rad. The skeletal dose estimate from 0.12×10^{-3} µCi/g of body weight was 1 rad of skeletal dose in 12 years. Another study¹³ reported that the relative sarcoma mortality is approximately linear as a function of dose, and the relative risk decreases as a function of time after injection. When beagles were given ²³⁹Pu at concentrations ranging from 0 to 3.30 µCi/kg of body weight in a single dose, the following results were obtained. The number of beagles who died of various causes was about 55% of the original number (158/285). Among those that died, the majority (58) died of osteosarcoma, whereas 39 died of other types of cancer. ⁹⁰Sr is a major fission product associated with nuclear fallout and radioactive wastes from nuclear power reactors. Raabe et al.¹⁴ summarized the effect of lifetime exposure of ²²⁶Ra and ⁹⁰Sr in beagles. ⁹⁰Sr induces predominantly bone marrow dyscrasias in comparison to bone cancer at a dose rate to the bone of 2–20 rads/day. Cancers of the soft tissue adjacent to the bone occurred in animals fed ⁹⁰Sr. This change was particularly important at low dose rates. However, ²²⁶Ra produced predominantly bone cancer at dose rates of 0.5–20 rads/day.¹⁴ No effects of these radionuclides were detected at skeletal doses below 0.5 rad/day.

A dose of ⁹⁰Sr (0.8 µCi/g of body weight) given i.p. produced predominantly osteosarcoma with a latent period of 320 days; however, at a lower dose (0.2 µCi/g of body weight), the predominant lesion was leukemia.⁷

¹³⁷Cs γ-radiation induced several types of tumors in mice.¹⁵ The relative risk of mortality for thymic lymphoma, myeloid leukemia, reticulum cell sarcoma, and lung tumors was predominantly linear as a function of dose (10–400 rads).

Relative risk of mortality from cancer decreased as a function of time after exposure. Some sex-related differences were observed.¹⁵ For example, ovarian tumors showed some unique features. Rates of mortality with ovarian tumors increased very sharply and nonlinearly for doses as low as 50 rads. In addition, the increased risk does not decline as a function of time after irradiation.¹⁵

³²P also induced a high incidence of osteosarcoma. Estimated skeletal doses were of the order of 10,000–20,000 rads, and in some cases the accumulated dose in rabbits has been as high as 40,000 rads. However, a single dose of 1000–1600 R produced a high incidence of

osteosarcoma in the rabbit. The latent period was greater than 1 year. The RBE of fission neutrons compared with γ -rays is about 3 for the induction of osteosarcoma, fibrosarcoma, and basal cell carcinoma.¹

D. BREAST TUMOR

The incidence of mammary tumors in rats and mice is dependent upon strain, tumor type, and irradiation conditions.¹⁷

It has been reported¹⁸ that the rat mammary tumor incidence appears to be linearly related to dose for both X- and γ -rays between 25 and 400 R. The RBE of fission neutrons for the production of mammary tumors was at least as high as 20; however, when the incidence was considered 11 months postirradiation, the RBE value¹⁹ of fission neutrons was about 8. The average induction time was 218 days. Female mice exposed to gamma radiation of 0.044–4.4 R/day for 10–15 months became sterile but did not develop mammary tumors. The dose rate may have slight or no effect on the incidence of mammary tumors, depending upon the type of tumors. Fractionation of 400–500 R of X-rays into as many as 32 exposures in a 16-week period produced no significant change in total incidence of tumor in rats, compared to single doses. However, an increase in the number of adenocarcinomas with fractionation was observed.²⁰

E. OVARIAN TUMOR

Most studies^{21–23} suggest that doses of 50–100 rads of X-rays produce the maximum tumor incidence in RFM and BALB/c mice. The incidence of tumors in these mice appears to be dependent upon dose rate;^{21–23} however, it has been reported²³ that one third of the effect of the dose rate was due to age-dependent loss of susceptibility to ovarian tumorigenesis, and two thirds was due to the dose rate effects. Because the sequence of events leading to the formation of tumor may be related to oocyte killing,^{24,25} a reduction in the incidence of ovarian tumor at low dose rates may be correlated, at least in part, with reduced killing of oocytes at low dose rates. It has been suggested^{25,26} that the decreasing dose rate affects the incidence of tumor in the following manner: (1) the size of threshold is increased from 12 to nearly 70 rads, and (2) the relationship between tumor incidence and dose is linear at a low dose rate, rather than squared.

F. UTERINE CARCINOMAS

A high incidence of uterine carcinoma was reported in rabbits after exposure (1.1–8.8 R/day in 8 hr, total dose 1400–2900 R). At a high exposure rate (8.8 R/day), the latent period was 36 months; at a low exposure rate (1.1 R/day), the latent period was 57.5 months. It is possible that this tumor results from a hormonal imbalance initiated by the irradiation.²⁷

G. SKIN CANCER

A high incidence of skin neoplasms in rats receiving whole-body proton irradiation (100–2500 rads) with a maximum tissue penetration of 1.4 mm was observed; 97% of the neoplasms developed from the skin. Doses as low as 200 rads produced a high incidence of tumor.²⁸ It has been suggested that irreparable follicular damage is an essential factor in the pathogenesis of radiation-induced skin tumors. Rats surviving a single whole-body exposure of 660 R showed a high incidence of neoplasm. After s.c. injection of ^{90}Y in mice, the incidence of malignant tumors was about 25% after doses of 10 and 30 μCi per mouse. The RBE of fission neutrons for the production of basal cell carcinoma¹⁶ is about 3.

H. TUMOR OF THE ALIMENTARY TRACT

Neither radiation-induced hepatoma, carcinoma of the stomach, nor adenocarcinoma of the small intestine in humans have been reported. In rats, the irradiation of the exteriorized small

intestine by X-irradiation (1400 R and above) produces a high incidence (67% of the survivors) of low-grade adenocarcinoma of the small intestine.²⁹ Clamping of the superior mesenteric vessels during irradiation, and i.p. injection of cysteamine before X-irradiation, provide protection against intestinal radiation death, but do not reduce the incidence of tumors among survivors. Fast-neutron irradiation (270 rads) also produces a high incidence of intestinal tumors.³⁰ An increased incidence of hepatoma in mice after chronic gamma irradiation has been reported.²⁷ An injection of 40–160 µCi of ¹⁹⁸Au produces 15% hepatoma in C57BL mice and 32% in RF mice, but cirrhosis occurred in 90% of the mice.³¹

I. THYROID CANCER

The injection of radioactive iodine increases the incidence of thyroid cancer in animals.³²⁻³⁵ A concentration of 32 µCi of ¹²⁵I produced 32% medullary thyroid carcinomas (control value 13%) and 43% follicular cysts (control value 4%) in rats,³² whereas ¹³¹I at low concentrations (3.2 µCi) produced about 15% medullary carcinomas and 53% of follicular cysts. Furthermore, it was observed that ¹³¹I doses of 8 µCi or higher prevented the natural incidence of these tumors.^{32,36} This may be due to the death of cells by irradiation.

J. PITUITARY TUMORS

In mice, destruction of the thyroid by ¹³¹I (200–300 µCi) may be followed by the development of pituitary tumors.³⁷ A smaller dose of ¹³¹I (30 µCi) destroys the thyroid if the mice have been on a low-iodine diet, but this dose does not cause pituitary tumors; however, when this dose of ¹³¹I is followed by whole-body irradiation (545 R), pituitary tumors develop. Thus, thyroidectomy enhances the induction of tumors by ionizing radiation. Thyroid implants or adequate thyroxine treatment prevents the induction of induced pituitary tumors, indicating further that the thyroid plays an important role in the development of pituitary tumor. Pituitary tumors appear to have very long latent periods.

K. ADRENAL TUMORS

It has been shown that adenomas of the adrenal medulla appeared to be more numerous in rats surviving longer than 1 year after an i.v. injection of radioactive polonium (1 µCi/kg of body weight or more) than in controls.

L. OTHER TUMORS

There is no association between radiation and tumors of the ureters, bladder, urethra, prostate, kidney, and extrahepatic bile duct in humans. Adenomas in the mouse kidney (25% of survivors) have been reported as a late effect of radiation.³⁸

II. EFFECT OF INTERACTION OF RADIATION AND CHEMICALS ON THE PROCESSES OF CARCINOGENESIS

Interaction between radiation and chemical carcinogens (benzopyrene, smoking, urathene, diethylstilbestrol, estrogen, ethionine, and procarbazine) has been extensively studied in animal models.³⁹⁻⁴⁸ A recent study shows⁴⁹ that the application of repeated 20-R exposure of X-rays during and following 7,12-dimethylbenz[*a*]anthracene (DMBA) enhances DMBA-induced Syrian hamster cheek pouch carcinogenesis. Irradiation prior to DMBA treatment is ineffective. Therefore, the time of irradiation is very important in determining the outcome of the effect of interaction between irradiation and DMBA.⁴⁹ It is unknown whether a similar phenomenon is observed when the interaction of radiation with other types of carcinogen occurs.

TABLE 18.1
Alterations in Oncogenes in
Radiation-Induced Cancer^a

Type of Cancer	Ras Oncogenes
Mouse lymphoma ⁵⁰	Activated c-ras
Mouse thymomas ⁵⁰	Activated k-ras
Rat skin carcinoma ⁵¹ and rat thyroid tumor ⁵²	Activated k-ras
Mouse thymomas (RF/J)	Mutated (k-ras and N-ras)
Mouse thymomas (C57BL/6J, ^{53,54} and canine leukemia ⁵⁵	Activated N-ras
Murine osteosarcoma ⁵⁶	Activated H-ras

^a Superscript numbers are reference numbers.

A. ALTERATIONS IN ONCOGENES IN RADIATION-INDUCED CANCER

The activated and mutated *ras* and overexpression of *c-myc* oncogenes⁸ are found in a variety of radiation-induced tumors in animals (Table 18.1); however, the incidence of abnormality in one or more of these oncogenes is seldom more than 50% of the tumor. Therefore, they cannot be considered as a primary target of radiation-induced carcinogenesis. The changes in oncogene expression reported thus far may reflect consequences of transformation in some cells rather than the cause of transformation. This is further confirmed by the fact that none of the cellular oncogenes identified thus far are able to transform normal cells upon transfection, although their viral counterparts do so. This is due to the fact that viral oncogenes contain strong transforming elements in their codon, whereas cellular oncogenes have weak transforming elements. One study has reported that activation of both *c-myc* and *k-ras* occurs at the later stage of carcinogenesis.⁵⁷

B. PREVENTION OF RADIATION-INDUCED CANCER

Although vitamins such as A, E, C, and β-carotene, as well as the mineral selenium, protect radiation-induced transformation *in vitro*, no studies have been performed to evaluate the role of these vitamins in radiation-induced cancer in animal models. Numerous studies have indicated that antioxidant vitamins and selenium reduce the risk of chemical-induced cancer in animal models.⁵⁸ One study has reported⁵⁹ that S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR 2721) reduced radiation-induced cancer in mice from 87 to 26%.

III. SUMMARY AND COMMENTS

Studies on animal carcinogenesis have provided useful information regarding the dose-effect relationships for several types of cancer. There have been some studies on the interaction of radiation with other cocarcinogens and tumor promoters; however, more data are needed on this problem. This is due to the fact that the numbers of tumor initiators and promoters in the environment are increasing, and therefore, they are likely to influence the incidence of cancer in humans. The effects of antitumor promoter substances, such as vitamins, have not been studied in radiation-induced animal carcinogenesis. Such studies are important for the possible chemoprevention program. It is true that studies of animal carcinogenesis are very costly and time-consuming; therefore, some of the hypotheses regarding radiation-induced cancers must be developed with *in vitro* models before testing them in animal models. Although alterations in some oncogenes such as *c-ras* and *c-myc* have been

observed in radiation-induced cancer in animals, none of them are considered as primary targets for carcinogenesis.

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Chapter 19

RADIATION CARCINOGENESIS: HUMAN MODEL

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I. INTRODUCTION

Results on radiation-induced cancer in humans are inadequate to establish a dose–effect relationship for all types of cancer. The primary sources of human data are from the following:

1. Populations of Hiroshima and Nagasaki who were exposed to single whole-body exposure during atomic bombardment in World War II; incidence of cancer — $1/10^4$ persons; primary cancers include leukemia, thyroid, breast, and lung.
2. Patients who were treated with ionizing radiation for non-neoplastic diseases; this generally involves local irradiation with fractionated doses: ankylosing spondylitis — primary cancer includes leukemia; tuberculosis — breast cancer; tinea capitis — thyroid cancer.
3. Patients who received diagnostic radiation; primary cancer includes leukemia.
4. Patients of cancer who survived more than 5 years after the completion of therapy involving radiation and chemotherapy; primary cancers include breast and thyroid cancers and leukemia.
5. Marshall islanders, exposed to atomic fallout; primary cancers include thyroid cancer.
6. Uranium miners; primary cancers include lung cancer.

Although the effect of a single dose of ≥ 10 rads of X-rays can be estimated with a certain degree of accuracy, it is difficult to estimate the carcinogenic effect of low doses of X-rays (< 10 rads) with fractionated exposures. Any estimation of the effect of the latter doses involves certain assumptions and extrapolations; therefore, any value of incidence of tumors at these low fractionated doses must be considered crude estimates.

Tissue culture and animal models suggested that several endogenous and exogenous factors may modify the incidence of tumors after irradiation. Some of these include tissues and organs, cellular origin, age, sex, total dose, dose rate, fractionation, LET, cocarcinogens, and tumor promoters. Some animal and human epidemiological studies suggest that the level of vitamins A, C, and E may be inversely related to the development of cancer.^{1-23,108-110} The diet and lifestyle also have an impact on carcinogenesis. It is likely that similar effects of vitamins,

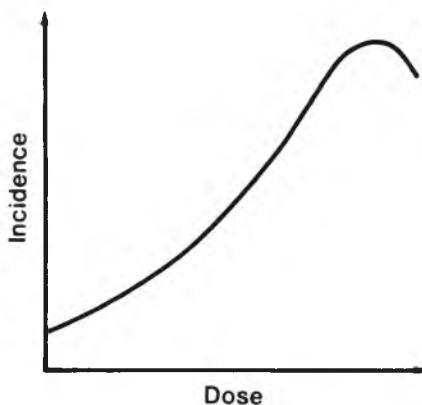


FIGURE 19.1. General dose-response curve.

lifestyle, and diet on radiation-induced cancer may exist. However, there are no human data at this time to support the above conclusion.

II. MODELS USED FOR THE ESTIMATION OF RISK

A. GENERAL DOSE-RESPONSE MODEL

The incidence of cancer increases as a function of dose (Figure 19.1). Cell killing at higher doses attenuates the incidence of cancer.²⁴

B. LOW AND HIGH DOSE-RESPONSE MODELS

There are three models:²⁴ linear-response (LR), quadratic-response (QR), and linear-quadratic-response (LQR), which have been used to estimate the effects of low doses of radiation on human carcinogenesis (Figure 19.2) in the BEIR report.²⁴ The values of risk depend upon the particular dose-response model. The value may vary at least by a factor of 2 (Table 19.1). The linear function of dose is primarily observed at low doses, whereas the proportional to square of dose is found at higher doses.

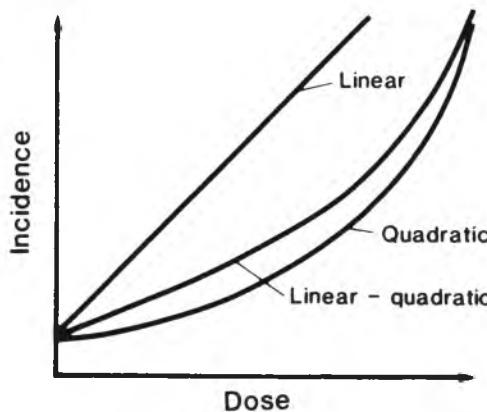


FIGURE 19.2. Redrawn and combined various dose-response curves from the BEIR report.²⁴

TABLE 19.1
Lifetime Risk Estimate of Leukemia and Bone
Cancer from Low-LET Radiation Doses

Exposure Condition	Model	Incidence (% of Normal Rate)	
		Male ^a	Female ^b
Single dose of 10 rads	LQ-L	2.8	2.3
	L-L	5.7	4.8
	Q-L	0.35	0.30
	LQ-L	15.0	13.4
	L-L	33.7	29.9
	Q-L	Not estimated	
Continuous exposure of 1 rad/year throughout lifetime	LQ-L		
	L-L		
	Q-L		

^a Normal rate = 9860 cases per 10^6 persons.

^b Normal rate = 8018 cases per 10^6 persons.

Data are summarized from Reference 24.

Below 400 rem (4 SV), the incidence of cancer is a linear function of dose for all cancers except leukemia. The incidence of leukemia reveals a linear-quadratic dose-response.

III. METHODS OF RISK ESTIMATION

A. ABSOLUTE RISK ESTIMATE

Absolute risk estimate is the excess of cancer above baseline which is caused by irradiation alone. Because it is impossible to separate radiation effect from that of other agents, it is not meaningful to express radiation cancer risk in absolute terms.

B. RELATIVE RISK ESTIMATE

Relative risk estimate is the excess of cancer risk above baseline which is due to interaction between radiation and other factors.

C. LIFETIME RISK ESTIMATE

The observations are made throughout the lifetime of the individual. The risk estimate is expressed as a number of cases per persons per Gy.

IV. LEUKEMIA AND LYMPHOMA

The first dose-effect relationship for human leukemia was estimated by Court-Brown and Doll.²⁵ They analyzed 13,352 patients who received fractionated doses of X-rays for the treatment of ankylosing spondylitis and postulated that (1) the incidence of leukemia increases with dose, and (2) there is no threshold dose. Other workers have questioned the linearity of the curve between 50 and 100 rads.

The incidence of leukemia among atomic bomb survivors in Hiroshima and Nagasaki has been analyzed²⁶ for the period of October 1, 1950 to September 30, 1966. The total air dose at 1000 m was 448 rads (neutron + γ -rays) for Hiroshima, and 925 rads (mostly γ -rays) for Nagasaki. The rate of leukemia increases sharply with dose in both Hiroshima and Nagasaki.²⁶

It also appears that the rates are higher in Hiroshima than in Nagasaki at almost every dose level, except for those who received a negligible dose of radiation. Moreover, the rates increase more steeply with increasing dose in Hiroshima than in Nagasaki. The difference in the incidence of leukemia between the two cities may be due to differences in the quality of radiation. The population of Nagasaki was exposed to gamma radiation, whereas the population of Hiroshima was exposed to γ -rays and neutrons. The RBE of neutrons, as compared with γ -rays, for the production of leukemia is about 5.0. The relative risk is greater in males than in females, especially in the high-dose region. Age has a marked and highly significant effect on the incidence of leukemia. The incidence is smaller among individuals 40 years or older. In Hiroshima, for persons in the low-dose (5–99 rads) region, there was an increased risk of acute lymphocytic, leukemia, other acute types, and especially chronic granulocytic leukemia. However, in Nagasaki, there was an increased risk in the low-dose region for acute and chronic granulocytic leukemia.

It was suggested that younger individuals are more sensitive to radiation-induced leukemia than older individuals. However, the recent BEIR (Biological Effects of Ionizing Radiation) report²⁴ suggests that the incidence of leukemia in the low-dose range, with fractionated exposures, increases with increasing age of the individual.

The BEIR report¹⁰⁷ suggests that radiation-induced leukemia generally appears within 10 years of exposure. In contrast, solid tumors have a longer latent period and continue to express, even after 30 years of exposure.

The relative risk of cancer among the survivors of the atomic bombing of Hiroshima and Nagasaki was examined from 1950 to 1985.²⁶ The relative risk factor for leukemia increases five-fold, whereas this varies from 1.2 to 3.0 for other cancers. Since more than half of the subjects entered in the study are still alive, this risk may increase in the future. Risk of leukemia is higher among persons who were under the age of 20 at the time of irradiation than those who were older than 20 years. Excess relative risk of leukemia per SV = 4.24–5.21. The excess of cancer risk mortality estimate is given in Table 19.2.

The threshold for leukemia is about 20 rads. This value has also been confirmed by another study.²⁷

There appears to be a prevalence of Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and multiple myeloma among atom bomb survivors of Hiroshima who received an estimated dose greater than 100 rads. Males who were less than 25 years of age at the time of the explosion showed relatively high risk.²⁸ Similar relationships between exposure and the subsequent development of lymphoma are not evident in the population of Nagasaki. Possible explanations for this discrepancy included defined physical differences in the

TABLE 19.2
Excess Cancer Risk Mortality Estimates, Lifetime per 100,000 Exposed Persons

Dose	Male			Female		
	Leukemia	Others	Total	Leukemia	Others	Total
Single, 10 rem continuous exposure	110	660	770	80	730	810
Exposure to rem/year between ages 18 and 65 years	400	2480	2880	310	2760	3070
Continuous lifetime exposure to 0.1 rem/year	70	450	520	60	540	600

Data summarized from BEIR.

TABLE 19.3
Effect of Low-LET Radiation on Excess Mortality
per Million on All Forms of Cancer²⁴

Exposure Condition	Excess Cases (% of Normal Rate, L-A Model)	
	Absolute Risk	Relative Risk
Single dose of 10 rads ^a	0.47	1.4
Continuous irradiation with 1 rad/year, lifetime accumulated dose: 75 rads ^b	2.8	7.2

^a Among 1 million persons of life-table age and sex in the U.S., 163,800 are expected to die from cancer.

^b Normal expectancy; 167,000/ 10^6 persons.

Data are summarized from Reference 24.

radiation spectra emitted by the two bombs, and genetic differences between the two populations at risk.

The BEIR report²⁴ has estimated the number of deaths due to all forms of cancer after 10^6 persons were exposed to a single dose of 10 rads or continuous irradiation with 1 rad throughout their lifetime (Table 19.3). Cancer incidence risk estimates are less firm than mortality incidence from cancer; therefore, the mortality data are commonly used. If 100,000 people of all ages are exposed to 10 rads (whole-body, simple dose), 800 extra cancer deaths may occur; however, 20,000 cancer deaths will occur in the absence of radiation.

From 1950 to 1974, the excess leukemia death per $10^6/\text{year/rad}$ (Kerma) was 2.33 in Hiroshima and only 1.46 in Nagasaki. Kerma (kinetic energy released in materials) is a unit of quantity that represents the kinetic energy transferred to charged particles by the uncharged particles per unit mass of the irradiated medium.

A preliminary report²⁹ suggests that nine cases of leukemia have occurred among 3224 men who participated in military maneuvers during the 1957 nuclear test explosion. This value is about three-fold higher than that of the expected value of 3.5 cases. The average latent period was about 14.2 years. Other workers have also reported³⁰ an increase in leukemia incidence among persons (10–14 years old at the time of exposure) exposed to fallout radiation in Utah. No excess risk has been found for other childhood cancers. The average dose was about 466.2 mrem. These authors have expressed the well-known cautions regarding the dose-effect relationships at these low doses.

V. LUNG CANCER

The more proximal regions of the bronchial tree are most radiosensitive to induction of bronchiogenic carcinoma by radiation. The incidence of lung cancer among miners working in mines involving radioactive substances is high.^{31–38} The average dose received at the site of the tumor was about 3000 rads and the average tumor induction time was about 17 years. An analysis³⁹ of the Hiroshima and Nagasaki data suggests that a dose greater than 128 rads increases the incidence of lung cancer by a factor of 2.

The induction of lung cancer depends heavily on age at exposure and duration of observation.⁴⁰ The excess death rate was 2.1 cases per 10^6 persons per year/rad for those individuals aged 35–49 years at the time of bomb exposure, and it was 4.9 cases for those 50 years of age

or older, when the values obtained for both Hiroshima and Nagasaki were combined. The absolute risk for excess cancer death among patients who received radiotherapy (average bronchial dose 197 rads, 20 rads per exposure) was about 2.8 cases per $10^6/\text{year/rad}$.^{26,41} The average age of these persons was 55 years. The risk estimate of miners is about 22–45 cases per $10^6/\text{year/rad}$.²⁶ If one considers the age-adjusted value of atomic bomb survivors and British ankylosing spondylitis to be about 3, the RBE of α particles is between 8 and 15. The RBE of the neutron is 5.

The excess risk for lung cancer could vary from 0 (<35 years at the time of diagnosis of cancer) to 7.0 cases per $10^6/\text{year/rad}$ (>65 years at the time of diagnosis of cancer).²⁶ The latent period is also dependent upon the age of the individual. The latent period appears to decrease with an increase of age.²⁶

Irradiated before age 15 years = 25-year latent period

Irradiated between 15 and 34 years = 15- to 20-year latent period

Irradiated at ≥ 35 years = 10-year latent period

Smoking greatly enhances the incidence of lung cancer.^{20,34,35} The BEIR report²⁶ estimates an increase in excess cancer risk by 50% among smokers; however, among nonsmokers, the excess risk is reduced by a factor of 6. Smoking also shortens the latent period.

The mean absorbed dose to lungs of survivors of Hiroshima was 8.8 rads of γ -radiation and 0.95 rad of neutrons.²⁶ The accumulated dose to bronchi of Canadian miners was about 4–9 rads.^{26,36} These doses would not significantly increase the incidence of lung cancer.

VI. BONE TUMOR

Primary cancers of the bone have been induced by high doses of therapeutic X-rays and by α -emitters.^{42–58} Osteosarcomas are the most common form of bone tumor. The incidence of osteosarcoma in the normal population is 1/200,000. Table 19.4 summarizes the results of several studies. The threshold skeletal dose from ^{224}Ra for the induction of osteosarcoma is 67–90 rads.⁵¹ A recent study⁵² shows that the threshold for accumulative radiation dose to induce bone tumor is 50–110 rads for mice, dogs, and humans. The accumulative dose required to give significant cancer risk is much less at lower dose rates than at higher dose rates, i.e., the higher dose rate produces more tumor. Based on German patients who received ^{224}Ra , the risk of bone sarcoma is 54 cases per $10^6/\text{patients per year/rad}$ after a single injection and 200 cases per $10^6/\text{rad}$ after multiple injection.⁵¹ The therapeutic radiation dose of 3000

TABLE 19.4
Effect of Irradiation on the Incidence of Bone Cancer

Type of Patients	Number of Patients	Dose	Number of Cases with Bone Cancer	References
Ankylosing spondylitis	14,000	1000 rads of X-ray to spine	1 in vertebrae, 4 in pelvis	44
German patients	900	>90 rads of ^{224}Ra , skeletal dose	54	51
German patients	1,000	<90 rads of ^{224}Ra , skeletal dose	2	48, 59
Dial painters	2,000	Mostly above 1000 rads	84	54

TABLE 19.5
Radiation-Induced Breast Cancer

Types of Patients	Doses	Relative Risk (10 ⁴ /rad/year)	References
Tuberculosis sanatoria, MA, U.S.	Unknown	1.7	67
Tuberculosis sanatoria, MA, U.S.	150 rads (1 rad per exposure), both breasts	2.1	68
Tuberculosis sanatoria, Ontario, Canada	Unknown	2.8	69
Acute postpartum mastitis, New York, U.S.	247 rads (1-10 exposures to both breasts)	2.0	70
Survivors of Hiroshima and Nagasaki	10 rads or more	1.7-1.8	71, 72
Benign breast disease, Sweden	Unknown	4.01	73

rads (fractionated) may induce osteosarcoma.⁶⁰ The latent period under conditions of continuous radiation could be as long as 52 years.⁵⁴

The risk of radiation-induced bone sarcoma is independent of age⁵⁶ and sex.⁵⁵

No increase in malignant bone tumor was found among the atom bomb survivors of Hiroshima and Nagasaki.⁵⁸ This may be due to the fact that the skeletal doses of survivors were much lower than needed to induce osteosarcoma.

VII. RISK OF LEUKEMIA AND BONE CANCER WHEN EXPOSED TO A SINGLE DOSE OF 10 RADs

The BEIR report²⁴ estimates that the lifetime excess death due to leukemia and bone cancer would be 274/10⁶ males and 186/10⁶ females. This represents 2.8% of the normal rate in males (9860 cases) and 2.3% of the normal rate in females (8018 cases) (Table 19.1). The normal death has been estimated to be about 20%.

VIII. RISK OF LEUKEMIA AND BONE CANCER WHEN EXPOSED TO 1 RAD/YEAR THROUGHOUT THE LIFETIME

The BEIR report estimates that the lifetime excess death due to leukemia and bone cancer would be 15% of normal in males and 13.4% in females.²⁴ These values could be doubled if the calculations are made using different models (Table 19.1).

IX. BREAST TUMOR

The female breast is very sensitive to radiation-induced carcinogenesis. A number of reviews have appeared on this subject.^{26, 61, 66} Table 19.5 summarizes the results of several studies. The following radiobiological principles are important:

1. The incidence of tumor is primarily related to dose at low dose levels.⁷⁴
2. Highly fractionated doses of X-rays are approximately as effective as a single dose.²⁶
3. Breast tissue may be more sensitive to radiation-induced carcinogens during proliferation of cells, such as during pregnancy.^{71, 75} Several animal studies show that hormonal factors may also modify the radiation-induced breast tumor.
4. The latent period does not depend upon dose, but rather upon age at exposure. The latent period for the survivors of an atom bomb of age 40-49 years was 5-9 years after

- exposure. The minimum latent period for women 25 years or older may be 5 years.²⁶ It has been estimated²⁶ that the maximum latent period may be greater than 30 years.
- 5. There are no firm data to establish the influence of age on the incidence of cancer.²⁶
 - 6. When women are exposed to a single dose of 1 rad,⁷⁶ the lifetime estimate of relative risk is 312 excess cases per 10^6 women for those irradiated at the age of 35, and 191 excess cases per 10^6 women for those irradiated at the age of 55.

X. OVARIAN TUMOR

Although X-rays induce both benign and malignant tumors in the ovaries, the radiosensitivity of the ovary for this particular criterion is relatively low.²⁶ Among 731 gynecologic patients treated with intracavitary radium or X-rays (700–2700 rads), the incidence of tumor was 3.1 times higher than the expected value.⁷ The latent period was about 10.1 years. Among survivors of Hiroshima who received 300+ rads, the induction rate was 0.6 ± 0.26 excess cases per 10^6 women/rad during a 12-year observation period, whereas it was only 0.04 ± 0.22 excess cases per 10^6 women/rad in Nagasaki.⁴⁰ The latter value was not significantly different. However, it is possible that the mean latent period for ovarian tumors is longer, and a rise in the radiation-induced rate of ovarian tumors could occur.²⁶ The rate of ovarian tumors in ankylosing spondylitis patients was similar to that of the expected value.⁷⁸

XI. CANCER OF THE UTERUS AND CERVIX

Recent results suggest that radiation induces carcinoma of both the uterus and cervix. It has been reported⁷⁷ that patients receiving radiation therapy with radium or X-rays exhibit 5.9 times higher incidence of uterine cancer than the expected value, with 4.9 cases per 651 patients and with the mean latent period of 9.7 years. The incidence of cervical cancer in these patients was only about 1.7 times higher than the expected value (6.5 cases per 651 patients) with the mean latent period of 8.5 years. Another study,⁷⁹ on patients treated with pelvic irradiation for metropathia hemorrhagica, reported that the incidence of uterine cancer was about 1.55 times higher than the expected value (10.3 cases per 2068 patients). It was estimated²⁶ that an absolute risk would be approximately seven excess deaths from uterine cancer per million exposed patients per rad for a follow-up of 5–19 years after 400 rads.

The data on survivors of Hiroshima and Nagasaki have failed to show any definite relationship between radiation dose and cancer of the uterus and cervix.²⁶ This may be due to the fact that the doses received by these survivors were not high enough to induce these types of cancer.

XII. SKIN CANCER

There are some studies that show that radiation induces skin cancer, primarily basal cell carcinoma. One case control study⁸⁰ shows that 19% of skin cancer patients and 4.5% of the controls had a previous history of radiation exposure. In another case control study,⁸¹ this one involving a Japanese population, 4.5% of skin cancer patients and 0.6% of controls had a history of previous radiation. The dose range in this study was 500 to 2000 R.

Table 19.6 summarizes the risk of skin cancer after therapeutic doses. The reasons for differences in various studies may, in part, be due to differences in observation periods, modes of radiation delivery, and total dose. It has been suggested that the increased fractionation and smaller dose per fraction may decrease the incidence of skin cancer.²⁶ The risk of skin cancer

TABLE 19.6
Effect of Radiation on Induction of Skin Cancer

Type of Patients	Dose	Absolute Risk (per 10 ⁶ /year/rad)	Observation Period (year)	Reference
Tinea capitis	700 rads to scalp	1.0	10-34	83
Enlarged thymus	330 rads to thymus	0.4	10-49	84
Uranium miners	100 rads to basal layer of skin	2.9		85
Survivors of atomic bomb	≥90 rads	No significant difference	19-21	86
Ankylosing spondylitis	1000-1500 rads	No significant difference	>9	87
Postpartum mastitis	280 rads	No significant difference	25	88

appears to increase with age. For example, the absolute risk per 10⁶ persons/year increases from 0 cases at 1-24 years of age to 3.047 cases at 40-44 years of age, and drops to 0 cases again at >44 years of age.^{26,82} The risk of skin cancer also appears to increase with the increase of the time interval after irradiation.^{26,82} For example, the absolute risk per 10⁶ persons/year increases at time intervals from 0 cases at 15-19 years to 4157 cases at 30-34 years. Excess risk may continue even after 45 years of exposure.^{26,85}

XIII. CANCER OF THE ESOPHAGUS

Radiation-induced cancer of the esophagus may be found, but the evidence is not very strong. The BEIR report²⁶ has estimated that the risk may be 0.39 excess cases per 10⁶/year/rad among survivors of Hiroshima. The risk may be higher for persons who were 35 years or older at the time of exposure.²⁶

XIV. STOMACH CANCER

Radiation-induced cancer of the stomach has been observed. The BEIR report²⁶ has estimated that the excess risk among patients with ankylosing spondylitis may be 0.59 excess cases per 10⁶ persons/year/rad. The dose estimate to the stomach was about 250 rads. Among the survivors of the atom bomb in Hiroshima, it was 1.57 excess deaths per 10⁶ persons/year/rad.²⁶ The mean dose to the stomach was 37 rads. The estimated risk for survivors of the atom bomb in Nagasaki was 1.05 excess deaths per 10⁶ persons/year/rad.

XV. CANCER OF THE COLON

The BEIR report estimated that the risk of radiation-induced colon cancer may be 1.7 ± 1.0 excess deaths per 10⁶ persons/year/rad among patients with ankylosing spondylitis. The average dose to the colon was about 57 rads. The risk estimate may vary from 0.1 to 1.7 excess deaths per 10⁶ persons/year/rad;²⁶ however, the value of 0.6 excess colon cancer cases per 10⁶ persons/year/rad, 15 to 25 years after exposure, was considered the best estimate.²⁶

XVI. LIVER TUMOR

Radiation also induces liver tumor in humans. Table 19.7 describes the lifetime estimate of risk of liver tumor among various radiation conditions. For the patients who received

thorotrust, it has been estimated that the risk for α -radiation would be 13 cases per 10^6 persons/year/rad, and for γ -radiation it would be 0.7 cases per 10^6 persons/year/rad.²⁶ The risk of ^{239}Pu -induced liver cancer is four times higher than that of ^{239}Pu -induced bone sarcoma.⁴⁸

XVII. TUMOR OF THE PANCREAS

Among 14,554 ankylosing spondylitis patients who received pancreatic doses of about 90 rads, an absolute risk was about 0.7 excess deaths from pancreatic tumor per 10^6 /year/rad during a follow-up period of 7–9 years.^{26,88} The most recent report on survivors of atomic bombs also suggests that there is a dose-response relationship for pancreatic cancer. Furthermore, it has been reported that there is an increase in incidence of pancreatic cancer among nuclear workers at the Hanford nuclear plant.^{89,90} The absolute risk estimate is about 10 excess deaths per 10^6 persons/year/rad.⁹¹ However, the BEIR report has suggested that these data are inadequate to establish a definite relationship between radiation dose and pancreatic cancer. More work is needed to confirm this observation. Excess mortality from pancreatic cancer (7 cases observed vs. 2.85 expected) has been reported in 923 patients who survived 5 years or more after radiation therapy of carcinoma of the cervix.⁹² Pancreatic cancers have also been reported in patients with lymphomas who received radium therapy.⁹³ Dose estimates for these observations are not available.

XVIII. TUMOR OF THE PHARYNX, HYPOPHARYNX, AND LARYNX

Some studies^{94,95} suggest that tumor of the pharynx and hypopharynx can be induced by therapeutic radiation doses (3000–6000 rads). The mean latent period is about 25 years (23–27 years). The tissue dose estimate is not available.

XIX. SALIVARY GLAND TUMOR

Both benign and malignant neoplasms of the salivary gland occur after therapeutic doses of irradiation.^{83,96,97} One study⁸³ has estimated that the risk of salivary gland tumor (doses less than 600 R in air) among children irradiated for benign thymus enlargement would be 5–10 excess salivary gland tumors per 10^6 persons/year/rad over a period of 20–40 years after irradiation. Other studies⁹⁸ have estimated 12 excess cases per 10^6 /rad for a 20-year observation period, and 16 excess cases per 10^6 /rad for a 15-year observation period.⁹⁹ The results obtained on survivors of the atomic bomb provided a risk estimate of 1 or 2 excess cases per 10^6 year/rad over a 19-year period.¹⁰⁰ The latent period for benign and malignant tumors is 13–25 years. The BEIR report²⁶ estimates that the induction rate for both benign and malignant

TABLE 19.7
Risk of Liver Cancers in Irradiated Persons

Type of Patients	Dose	Type of Radiation	Lifetime Risk Estimate (Cases per 10^6 Persons/rad)	Reference
Thorotrast injected	25 rads/year	α particles	300	88, 26
		γ -rays	15	
Hiroshima	10 rads to liver	Neutron	443	26, 40
		γ -rays	231	
^{239}Pu	40 rads	α particles	300	26, 48

tumors is no more than 10 excess cases per 10^6 children/rad over a 20-year follow-up period; however, the rate may increase as the time interval after irradiation increases.

XX. THYROID TUMOR

From the data on the survivors of atomic bomb, it was estimated that a dose of 50 rads or more produces thyroid tumors. Recent studies^{101,102} estimate that a dose of 6.5–7 rads may produce thyroid cancer. The BEIR report²⁶ estimates that the risk of thyroid cancer is about 1.6–9.3 cases per 10^6 /year/rad. The X-irradiation is 10–80 times more effective than β -irradiation.¹⁰³ This estimate is based on animal studies. However, β -irradiation is more effective in inducing hyperthyroidism. Radiation-induced thyroid cancers are mostly the papillary and follicular types.^{103,104} The BEIR report²⁶ suggests that females are 2.3 times more sensitive than males. The incidence among females of Jewish heritage may be 17 times higher than non-Jewish females. There is no relation between lower doses and the latent period. The latent period may vary from 10 years to over 35 years after irradiation.²⁶ Age was initially considered an important factor in radiation-induced thyroid cancer, but the BEIR report indicates that age may be a weak influence.²⁶ Thyroid adenoma may have a higher incidence than thyroid carcinoma with smaller doses of radiation.²⁶ The incidence of thyroid adenoma is about 12 cases per 10^6 /year/rad, which is about three times higher than that of thyroid carcinoma.¹⁰⁰

XXI. BRAIN TUMOR

Radiation also induces brain tumors. The case control studies of children who received an average dose of 0.8 rad *in utero*, and who died of CNS tumor during 0–14 years of age, suggest that the absolute risk was about 6.1 excess deaths per 10^6 /year/rad.¹⁰⁵ Another study estimates the absolute risk of about 6.3 excess deaths per 10^6 /year/rad.¹⁰⁶

In another series involving 140 rads to the brain of children (8 years old) for the treatment of ringworm of the scalp, the absolute risk was about 1.3 excess deaths per 10^6 /year/rad on the basis of a follow-up period of 15–34 years. The minimum latent period was 5 years, but tumors were observed more than 25 years after irradiation.⁸²

Table 19.8 describes ICRP estimates of radiation-induced cancer in whole and working human populations. For the whole population, slightly high risk estimation may be due to the fact that younger individuals show increased sensitivity to radiation. Table 19.9 describes RBE values for radiations of high-LET for carcinogenesis.

TABLE 19.9
RBE Values of High-LET Radiation
for Carcinogenesis

High-LET Radiation	RBE Values
Helium-4	5
Carbon-14	12
Neon-20	18
Argon-40	27

Taken from Fry et al., *Radiat. Res.*, 104, 188, 1985.

TABLE 19.8
ICRP Estimate of Risk of Radiation-Induced
Cancer in Humans (Cases per 10⁴ per SV)

Type of Population	High Dose at High Dose Rate	Low Dose at Low Dose Rate
Working Whole	8 10	4 5

Summarized from ICRP Publication Co., 1990.

XXII. ONCOGENES AND HUMAN TUMORS

Several oncogenes are identified in human tumors, but none of them appear to be causative agents. For example, point-mutated *c-ras* is associated with several human cancers including colon and bladder; however, this defect in *c-ras* oncogene is present only in up to 50% of tumors. Similarly, other oncogenes — such as rearranged *c-abl* in myelogenous leukemia, rearranged *c-myc* in Burkitt lymphoma, and overexpression of *c-myc* and *c-erb* in some tumors — are elevated, but incidence of any of the above gene defects in any given human tumor is never more than 50%. Furthermore, none of these cellular oncogenes transform normal human cells upon transfections. Therefore, they cannot be considered sufficient for the induction of tumors in the human.

The presence of antioncogenes or suppressor gene, *rb*, was first identified in retinoblastoma. The *rb* gene is located on chromosome #13 and is considered recessive. Mutation on the *rb* gene of one chromosome is not sufficient to cause cancer; however, when the loss or mutation of a normal allele on the other chromosome #13 occurs, cancer is formed. Therefore, the normal *rb* gene located on chromosome 13 can be considered an antioncogene, because *rb* presence prevents the formation of retinoblastoma. Patients with retinoblastoma have no normal copy of *rb* genes. Patients with retinoblastoma have a high risk of developing osteosarcoma following radiation therapy. Irradiation may cause loss or mutation of suppressor genes, which otherwise prevents the development of osteosarcoma in the absence of irradiation. The function of the *rb* gene as a suppressor gene could not be confirmed by direct experiments because the *rb* gene failed to convert cancer cells to normal cells upon transfection.

One of the most widely known studies, with respect to cancer and growth regulation, is the p53 gene, which codes for a 53-kDa nuclear phosphoprotein involved in the control of cell proliferation. Although a wide spectrum of mutations has been observed in human tumors, such as lung, esophagus, breast, liver, and brain, the incidence of this defect never exceeds more than 50% in any tumor. Therefore, p53 gene mutations play no direct role in carcinogenesis. Chromosome aberrations are the only consistent findings in all human cancers. Such aberrations lead to random changes in oncogene expression. The key genes or oncogenes that play a crucial role in initiating carcinogenic events have not yet been identified.

XXIII. PROPOSED STRATEGY FOR CANCER PREVENTION

Radiation-induced cancer is indistinguishable from cancer induced chemically or spontaneously. Therefore, the current proposed cancer prevention strategies could be adopted for radiation-induced cancer: the National Cancer Institute has recommended modification in diets and lifestyle. These recommendations include a low-fat and high-fiber diet, which is rich

in fresh fruits and vegetables. In the current recommendation the total fat calories can be reduced to 20% (1 g fat = 9 calories). Excessive amounts of fat can act as tumor promoters; in addition, they produce high levels of prostaglandins, which are immunosuppressive. High fibers can bind bile acid, cholesterol, and some mutagens that are formed in the GI tract; they are eliminated through feces. The fermentation of fiber by endogenous bacteria generates millimole levels of butyric acid, a 4-carbon small fatty acid, in the lower intestinal tract. Sodium butyrate has been shown to reduce the growth of several types of cancer. Sodium butyrate may be one of the mechanisms involved in the cancer-protective effect of a high-fiber diet, and this mechanism of protection could be applicable to all cancers. In addition, the consumption of cured meat (rich in nitrite), smoked foods, and pickled foods should be reduced. The lifestyle change recommendation includes cessation of tobacco smoking and chewing, reduction in consumption of alcohol and caffeine, and adoption of habits of regular exercise and reduced stress.

In addition to modifications in diet and lifestyle, some scientists have recommended a moderate supplement of those dietary vitamins (vitamin A, β -carotene, vitamin C, and vitamin E) which are shown to reduce the risk of cancer *in vitro* and in animal models. This recommendation is based on the following rationales:

1. All *in vitro* and animal studies on carcinogenesis have been performed on supplemental vitamins, and they support the role of vitamins in cancer prevention.
2. Several but not all human epidemiological studies have confirmed that higher levels of vitamins such as β -carotene and vitamins A, C, and E reduce the risk of cancer.
3. A few intervention trials have further confirmed the role of these vitamins in cancer prevention.
4. Because all foods contain varying levels of manmade and naturally occurring mutagens and naturally-occurring antimutagens, the risk of cancer will depend upon the relative intake of these two groups of substances.

To increase the intake of antimutagenic substances, a supplement of vitamins — in addition to a balanced diet — would be necessary for a maximum reduction in cancer risk.

XXIV. CONCLUSION AND COMMENTS

If one examines the radiation carcinogenesis after low doses of radiation, it becomes clear that only three types of tumors, namely leukemia, breast cancer, and thyroid cancer, can be induced by diagnostic doses of radiation (Table 19.10). The induction of other types of tumors (lung cancer, bone tumor, ovarian tumor, cancer of the cervix and uterus, skin cancer, stomach cancer, colon cancer, brain tumor, and liver tumor) generally requires therapeutic radiation doses. The relative risk for these tumors is generally one to six cases per 10^6 /year/rad. All tumors in fetuses, except leukemia, require a threshold dose of low-LET radiation. The latent period is generally longer than 10 years except for leukemia (generally within 10 years). Fractionation of a radiation dose increases the incidence for certain types of tumor. RBE of the neutron for leukemia is about 5. In addition to ionizing radiation, there are other cocarcinogens, tumor promoters, and antitumor promoters outside and inside the body environment. Furthermore, diet and lifestyle have a major impact on cancer incidence. These agents must influence the incidence of radiation-induced cancer in humans. Therefore, when estimating the hazards of radiation on human cancers, the effect of other carcinogens and diet and lifestyle factors must be considered. None of the previous epidemiological studies on radiation-induced cancer has considered the impact of recently identified dietary confounding factors. There is no direct evidence that the cellular oncogenes or antioncogenes play a role in any human carcinogenesis. The overexpression of

TABLE 19.10
Relative Risk of Cancer after a Single
Dose of Radiation

	Threshold Dose (rads)	Relative Risk Cases per 10 ⁶ /year/rad
Leukemia	20	3.1
Breast	≤1	6.6-8.7
Thyroid	6-7	1.6-9.3

Note: High incidence of leukemia is observed after exposure of fetuses with diagnostic doses of less than 10 rads.

cellular oncogenes or mutated oncogenes, which are often noted in some human tumors, may be the consequence of cancer rather than the cause of it. An interim guideline for reducing the risk of radiation-induced cancer has been proposed.

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Chapter 20

RADIATION CARCINOGENESIS: SECONDARY NEOPLASM AFTER TUMOR THERAPY

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I. INTRODUCTION

The aggressive treatment of human tumors involving ionizing radiation and chemotherapeutic agents has produced long-term survivors of certain types of cancer. Because almost all chemotherapeutic agents and ionizing radiation are known to be mutagenic and carcinogenic, the possibility of developing secondary neoplasms among long-term survivors of cancer is not an unexpected observation. Indeed, an increasing number of secondary malignancies with shorter latent periods is being reported, especially among children. There are instances where either ionizing radiation or chemotherapeutic agents alone have induced secondary malignancy; however, in most instances, the appearance of a second malignancy among long-term survivors of cancer is the result of the net effect of both ionizing radiation and chemotherapeutic agents. Experimental data using tissue culture have already suggested that the combined treatment of hamster embryo cells with ionizing radiation and benzo[*a*]pyrene dramatically enhances (nine-fold) the frequency of transformation. Therefore, the increased incidence of secondary malignancy among long-term survivors of cancer treatment is not surprising. In addition to malignancy, there are other non-neoplastic changes that are being observed in an increasing number. The purpose of this chapter is to describe the most recent information on the long-term consequences of current tumor treatment modalities.

II. SECOND MALIGNANCIES AMONG SURVIVORS OF HODGKIN'S DISEASE

The development of a second malignancy is becoming increasingly evident among the long-term survivors of Hodgkin's disease. The first reports of such a hazard appeared several

years ago,^{2,3} however, only recently have the hazards of current treatment modalities involving radiation and chemotherapy been realized.³⁻⁸ This is not surprising, because almost all agents used are carcinogenic and/or mutagenic. Indeed, the carcinogenic and noncarcinogenic effects of these agents have been observed in humans.⁹ A study reports⁸ that incidence of solid tumor among survivors of radiation therapy alone was about 14.9%, with no incidence of leukemia; however, the incidence of leukemia among survivors of chemotherapy alone was 5.5% with no incidence of solid tumor within 10 years of observation (Table 20.1). The reasons for such a differential effect between radiation and chemical treatment on the type of tumor are unknown. Both solid tumors and leukemia were observed among survivors of treatment involving radiation and various combinations of chemotherapeutic agents (Table 20.1). The average incidences of solid tumor and leukemia were 6.2 and 3.5%, respectively. Thus, the incidence of total malignancy among the survivors of Hodgkin's disease was about 9.7%. Obviously, both ionizing radiation and chemotherapeutic agents are responsible for these effects.

III. BASAL CELL CARCINOMA AS A SECOND MALIGNANCY

The incidence of basal cell carcinoma as a second malignancy appears to be very high, especially among children. The data from various studies have been summarized in Table 20.2. Although the latent period in several instances appears to be within 5 years, the median latent period appears to be 21 years. One patient with nevoid basal cell carcinoma was treated with radiation therapy (88 rads per visit, a total dose of 704 rads) to the hand at 5 years of age. At age 28, this patient developed multiple basal cell carcinomas of the palm and dorsal of the hand.¹³

Radiation-induced osteosarcoma has been reported in several patients.^{14,15} In one study,¹⁵ 4 of 76 patients developed osteogenic sarcomas. One was treated with megavoltage radiation

TABLE 20.1
Incidence of Second Malignancy in Hodgkin's Disease Within 10 Years After Treatment Involving Radiation and/or Chemotherapy

Treatment Modality	Type of Malignancy	Incidence of Second Malignancy within 10 Years (% of Survivors)
Radiation therapy*	Solid tumor	12
Radiation therapy	Leukemia	0
Chemotherapy	Solid tumor	0
Chemotherapy	Leukemia	5.5
Radiation therapy	Solid tumor	0
Chemotherapy (MOPP) ^b	Leukemia	5.4
Radiation therapy	Solid tumor	2.1
Chemotherapy (MABOP) ^c	Leukemia	3.8
Radiation therapy	Solid tumor	0
Chemotherapy (ABVD) ^d	Leukemia	0
Radiation therapy	Solid tumor	12
Chemotherapy (other)	Leukemia	2.9

* Radiation therapy = 3000-5000 rads.

^b MOPP = mustine, vincristine, procarbazine, and prednisone.

^c MABOP = MOPP with adriamycin and bleomycin substituted for procarbazine.

^d ABVD = adriamycin, bleomycin, vinblastine, and dacarbazine.

Data from Valagussa, P., Santoro, A., Kenda, R., Fossati-Bellani, F., Franchi, F., Banfi, A., Rieke, F., and Bonadonna, G., *Br. Med. J.*, 280, 216, 1980.

TABLE 20.2
**Incidence of Basal Cell Carcinoma Among Long-Term
 Survivors of Radiation Treatment**

Number of Treated Patients	Latent Period	Basal Cell Carcinoma as a Second Malignancy (% of Total Patients)	Reference
13 patients of medulloblastoma treated with radiation	6 months to 3 years	100	10
3 patients treated with radiation	1-4 years	100	10
1 child treated with radiation shortly after birth	5 years	100	12
1 child received radiation treatment at the age of 5	23 years	100	13

alone (6000 rads in 30 fractions, 5500 rads in 22); the other three received cyclophosphamide and vincristine, plus X-irradiation. The latent period varied from 19 to 76 months. Chemotherapeutic agents appear to play an important role in the increased risk of second neoplasms.¹⁶ Several studies have reported that X-irradiation alone would increase the risk of second neoplasms among long-term¹⁷⁻²⁸ survivors of childhood malignancies. Orthovoltage irradiation also induced second neoplasms in many sites.¹⁸ Two cases of radiation-induced sarcoma have been reported in another study.²⁹ Nine patients who received therapeutic doses (3200–5500 rads) developed new tumors at the site of irradiation.³⁰ These include thyroid adenoma (latent period 21 years), fibrosarcoma of the sacrum (latent period 4 years), carcinoma of the pancreas (latent period 7 years), breast carcinoma (latent period 10–41 years), leimyosarcoma (latent period 24 years), adenocarcinoma (latent period 26 years), and carcinoma of the uterus (latent period 11 years). It has been reported³¹ that two patients who were previously treated for malignant disease by surgery and ⁶⁰Co-irradiation (3600–4000 rads) developed melanoma in tattoo sites used for marking a radiation field. The exact reasons for this are unknown.

IV. EFFECT OF TUMOR THERAPEUTIC AGENTS ON THE INCIDENCE OF NON-NEOPLASTIC DISEASES

The adverse effects of tumor therapeutic agents (X-irradiation and chemotherapeutic agents) on the reproductive system of long-term survivors of childhood malignancies have been reported.³²⁻³⁴ The overall ovarian failure incidence is 12%,³⁴ however, the incidence of failure is dependent upon the amount of ovarian area irradiated (Table 20.3). Gonadal failure has also been reported in male subjects of all ages after treatment with tumor therapeutic agents.³⁵

TABLE 20.3
Effect of Tumor Therapeutic Agents on Ovarian Failure

Amounts of Gonad Irradiated	Total Number of Subjects	Ovarian Failure (%)
Both ovaries (ovary dose 320 rads)	25	68
One or both ovaries at the edge of treatment field (ovarian dose 290 rads)	35	14
One or both ovaries outside the treatment field (ovarian dose 54 rads)	122	0

Data are summarized from Razis, D.V., Diamond, H.D., and Craver, L.F., *Am. J. Med. Sci.*, 238, 327, 1959.

TABLE 20.4
Cancer Incidence After a Therapeutic Dose of ^{131}I

Type of Cancer	Dose (cGy)	Value Higher than Normal (%)
Lung	7	32
Kidney	5	39
Stomach		33
Thyroid	>10,000	29
Parathyroid	No estimate	78
Brain	No estimate	30

Data are summarized from the work of Holm et al.³⁶

V. RISK OF CANCER FOLLOWING ^{131}I THERAPY

The overall cancer risk among patients who received ^{131}I therapy for hyperthyroidism was only 6% greater than that expected in general populations; however, the risk of individual cancer was higher than that of overall value³⁶ (Table 20.4). One study reported that the incidence of breast cancer among women treated with ^{131}I for hyperthyroidism was 90% greater than that found in the general population;³⁷ however, another study found no such increase in breast cancer risk.³⁶

VI. SUMMARY AND COMMENTS

The aggressive treatment of human tumors, involving ionizing radiation and chemotherapeutic agents, has produced long-term survivors for certain types of cancer. Because these tumor therapeutic agents are themselves mutagenic and carcinogenic, the increased incidence of a second malignancy among long-term survivors is not an unexpected observation. In addition to cancer, treatment-induced non-neoplastic diseases have also been observed among the long-term survivors. Therefore, the approaches to long-term management of human tumors must exclude the extensive use of mutagenic and carcinogenic substances. Increasing numbers of nontoxic compounds such as vitamins and maturing factors, including cyclic nucleotides, are becoming available for the management of tumors. It should be emphasized that until we have developed new approaches to the management of tumors, the use of current treatment modalities involving chemotherapeutic agents and ionizing radiation must be continued, in spite of the fear of developing neoplastic and non-neoplastic diseases as a long-term consequence of treatment.

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Chapter 21

OTHER LATE EFFECTS: AGING, CATARACT, AND APLASTIC ANEMIA

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I. INTRODUCTION

In addition to inducing cancer, ionizing radiation has been shown to cause several types of non-neoplastic changes including aging, cataract, aplastic anemia, and delayed necrosis in radioresistant tissue. The last subject has been discussed in chapters dealing with the effect of irradiation on organ systems. This chapter will discuss the effect of irradiation on aging, cataract, and aplastic anemia.

II. EFFECT OF IRRADIATION ON AGING

A. ANIMAL MODEL

It has been established that radiation accelerates the aging process in animals, but it remains controversial in humans.¹⁻⁷ It has been shown^{3,4} that the radiation-induced life-shortening phenomenon is more pronounced in mice irradiated early in life than in mice irradiated at a later stage. In the dog, whole-body exposure of 100 and 300 R causes a reduction in the life span by 9.5 and 20.7%, respectively. This represents a loss of about 6.7% of control per 100 R. Fractionated exposure reduces the effect of irradiation on aging.^{8,9} Anoxia and SH compounds also reduce the effect of irradiation on the life span of animals.^{10,11} The effect of bone marrow treatment immediately after X-irradiation, or the shielding of blood-forming organs before irradiation, does not reduce the life-shortening effect of irradiation. The RBE value¹² of a neutron for the criterion of life-shortening is about 10.

Radiomimetic agents such as nitrogen mustard (3.7–4.5 mg/kg of body weight) and triethylene melamine (3–4 mg/kg of body weight) also reduce the life span of mice.¹³ The following results were obtained:

Treatment	Mean Survival Time (days)
Control	638
Nitrogen mustard	561
Triethylene melamine	508
500–600 R	396

The hypothesis has been proposed that radiation causes life shortening by accelerating the normal aging processes. The opposite suggestion has been made: radiation does not accelerate the aging process but reduces life span by accelerating the occurrence of non-neoplastic diseases.¹⁴

B. HUMAN MODEL

The effect of irradiation in reducing the life span in humans has not been fully established. An analysis was made of 492 women who received 0.02–10 µCi radium per kilogram of body weight. There was no evidence that the above radium doses caused any life shortening.⁵ However, one group of investigators⁶ estimated that the reduction in survival time after whole-body exposure may be 1 day/R at a dose rate of 0.5 R/day, whereas another study⁷ estimated that the reduction in life span may be about 15 days/R. The extrapolation of mouse data to humans provides an estimate of reduction in life span of 1.2 days/rad.¹⁵ Another method yields the value of about 1 day/rad.¹⁶

C. TISSUE CULTURE MODEL

The monolayer culture of human diploid fibroblasts has a finite life span of about 50 passages. This model has been used to establish whether radiation accelerates aging processes. It has been reported that X-irradiation¹⁷ and thermal neutron¹⁸ irradiation reduce the life span of human fibroblasts in culture.

III. APLASTIC ANEMIA

Aplastic anemia in humans has been observed after radiation therapy. It has been reported¹⁹ that deaths due to aplastic anemia in patients receiving 112–3000 R for the treatment of ankylosing spondylitis were approximately 30 times higher than expected. It has been estimated²⁰ that the number of observed deaths due to aplastic anemia was 17 times greater among American radiologists than expected. A recent report on survivors of the atom bomb suggests²¹ that the relative risk of aplastic anemia is about 1.8, which was not considered statistically significant. The difference in results may be attributed to the following factors: (1) in the case of the atom bomb survivors, radiation was delivered in a single dose, and only those receiving a sublethal dose survived—whereas in the case of radiation therapy, large doses are given in several fractions; and (2) differences in racial background may be important, since idiopathic aplastic anemia is relatively more frequent in the Japanese than in Europeans and Americans, whereas secondary aplastic anemia induced by various drugs, especially chloramphenicol, is frequently found among Europeans and Americans.²² Table 21.1 shows the relative risk of aplastic anemia in various groups of individuals.

Experiments with animals also have shown that radiation produces aplastic anemia. Rats exposed to nine doses of 150 rads (once every 4 weeks) developed permanent anemia 100 days

TABLE 21.1
Comparison of Risk for Aplastic Anemia Among Irradiated Persons

Subject	Type of Radiation	Dose	Relative Risk (obs/exp)	Reference
Ankylosing spondylitis	X-rays	112 to more than 3000 R	29.4	19
American radiologist	X-rays	Unknown	17.4	20
Atom bomb survivors Hiroshima and Nagasaki	Gamma and neutron	1–100 rads	1.8	21

or more after the final radiation exposure.²³ The permanent anemia was not cured by treatment with five daily doses of either 5 or 25 units of erythropoietin. It has been suggested that permanent anemia is caused by a reduced capability of cellular proliferation due to accumulation of residual injury in stem cells.

IV. RETINAL DYSPLASIA

Retinal rosettes and dysplasia were observed in irradiated human fetuses.²⁴ The pathology of the dysplastic lesion includes necrosis in the neuroblastic layer, resorption of the necrotic foci, and rearrangement and further differentiation of viable cells.²⁵ When 2-day-old dogs were exposed to 170 R of gamma radiation, dysplastic changes were seen.²⁶ These include necrosis, lysis, migration, and tubule formation. Continuing necrosis of cells in the dysplastic neural retina for as long as 6 months after irradiation ultimately produced atrophic changes.

V. CATARACT FORMATION

Radiation causes cataract formation in humans and animals. Radiation damages epithelial cells, which results in lens fiber disorganization. The degree and duration of this process of lens fiber disorganization determine the degree of cataract. The severity and rate of progression are dose-dependent, and the latent period is inversely related to the dose. Fractionation of X- and Gamma-radiation doses tends to reduce the cataractogenic effect of radiation and delays the onset of cataracts. The dose-response curve for the radiation-induced cataract in humans is the sigmoid type with an apparent threshold dose.^{27,28} The threshold dose in humans is 200–500 rads (single dose) or 1000 rads (fractionated dose). The latent period is about 10 months. Young and old lenses have similar sensitivities for the induction of cataracts. The rate of progression of the disease and the extent of opacification may be dependent upon age. At doses of 300–900 rads, opacities occurred earlier in younger lenses, but the rate of progression of disease was greater and the extent of opacities was more severe in adult lenses. At doses greater than 900 rads, cataract occurred sooner and progressed more rapidly in young lenses.

An exposure of 1150 R causes 100% cataract formation. The latent period for cataract production varies from 10 months to 35 years with an average of 2–3 years.

The RBE of neutron for the production of cataract is dependent upon neutron energy.²⁹⁻³¹

Energy	RBE Value
14 MeV (fast neutron)	10
3 MeV	5
0.3 MeV	30
Fission neutron	50

Topical ocular applications of dimethyl sulfoxide (DMSO) before irradiation in a concentration range of 10–100% completely prevents cataract formation in mice,¹⁴ but it results in radiosensitization of the cornea. The radiosensitizing effect of DMSO on the cornea may be due to a nonspecific stress on the irradiated cornea, because DMSO produces no corneal lesion in nonirradiated animals. DMSO is ineffective in protecting mice against radiation-induced cataract when given after exposure. The mechanism of DMSO protection may involve hypertonicity to some extent, and it may also involve alteration in the epithelial cell metabolism.

VI. GROWTH IMPAIRMENT

Among survivors of Hodgkin's disease, growth impairment is one of the most serious late effects of ionizing radiation when given before the completion of skeletal growth of a child.³⁴ Retardation of spinal growth was most marked in patients receiving doses of greater than 35 Gy to the axial skeleton who were less than 6 years of age at the time of exposure.

VII. SUMMARY AND COMMENTS

Ionizing radiation induces non-neoplastic diseases in animals and humans. These include aging, aplastic anemia, retinal dysplasia, cataract formation, and delayed necrosis in radioresistant organs. Although it has been established that X-irradiation reduces the life spans of animals, it is not certain whether ionizing radiation produces a similar effect in humans. Recent studies using monolayer cultures of human fibroblasts have shown that irradiation of cells reduces their life span in culture. Chronic irradiation also causes aplastic anemia in humans and animals. Retinal rosettes and dysplasia are also observed in irradiated human fetuses.

Radiation also causes cataract formation in humans and animals. The threshold dose in humans is about 200–500 rads (single dose) or 1000 rads (fractionated dose). The latent period varies from 10 months to 35 years with an average of 2–3 years. Neutrons are more effective than X-rays in producing cataracts in animals. The RBE value of a neutron for the production of a cataract is dependent upon the energy of the neutron. The RBE value of the fast neutron is 10, whereas for fission neutrons it is 50.

Topical ocular application of DMSO before irradiation completely prevents cataract formation in mice; however, it results in radiosensitization of the cornea. The mechanisms of the diverse effects of DMSO on the lens and the cornea are unknown.

Whether ionizing radiation accelerates normal aging processes remains to be ascertained. The tissue culture model of normal human fibroblasts may provide a useful model to study this problem.

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Chapter 22

MAXIMUM PERMISSIBLE DOSE

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I. INTRODUCTION

The extensive use of atomic energy in various branches of the national economy, technology, science, biology, and medicine has made the study of radiation injuries an urgent problem. The critical question is, therefore, What is the minimum dose that could be considered safe? There is no radiation dose known as "safe" or "harmless."

To understand the significance of the maximum permissible dose (MPD), it is necessary to know the background radiation to which man has been exposed throughout his evolution. The level of natural background varies as a function of elevation and also as a function of deposits of radioactive nuclides in the vicinity (Table 22.1).

II. HISTORY OF MAXIMUM PERMISSIBLE DOSE (MPD)

The concept and value of the MPD have markedly changed since they were proposed in 1925. The First International Congress of Radiology, in 1925, established the International Commission on Radiological Units (ICRU); in the same year, the MPD value for X-rays and radium was suggested to be one tenth of an erythema dose per year (estimated exposure 0.2

TABLE 22.1
Level of Natural Background Radiation

Location	Rems/Year
New York (sea level)	0.1
Denver (5280 feet elevation)	0.15-0.2
Kerala, India (radioactive mines area)	1.5-5.0

R/day). The threshold erythema dose was very unsatisfactory as a quantitative unit of radiation exposure, because the magnitude of radiation-induced erythema depended upon several factors. These include the texture of the skin, thickness of the epidermis, age, color of the skin (black, white, blonde, and brunette), energy of radiation, and time of exposure. In addition, the identification of skin changes as a threshold erythema dose was variable from one person to another. The values of the MPD have continued to decrease as our knowledge of the radiation effects have increased. Table 22.2 shows the change in MPD values for radiation workers since they were proposed in 1934. The responsibility for recommending units for radiation exposures has been assigned to the advisory groups composed of persons experienced in the use of ionizing radiation. The International Commission on Radiological Protection (ICRP) was formed in 1928 to make a recommendation for the MPD. The National Council on Radiation Protection (NCRP) was formed in 1929 to interpret and implement the recommendations of the ICRP for the U.S. The Federal Radiation Council (FRC) was established in 1959 to advise the President of the U.S., to provide guidance in the formulation of radiation standards, and to provide a federal policy concerning human radiation exposure. The recommended MPD values for various categories of human populations are given in the *Handbook of the National Bureau of Standards*. Some of the pertinent references are listed at the end of the chapter.¹⁻¹⁷

ICRP has arbitrarily replaced the relative biological effectiveness (RBE) value with radiation weighting factor (WR), which is sometimes also referred to as radiation quality factor. The WR factor for each radiation is described in Table 22.3.

A. EQUIVALENT DOSE

In radiological protection recommendations, the equivalent dose is measured in SV; the absorbed dose is measured in Gy. When equivalent dose is expressed in rem, the absorbed dose is in rad.

TABLE 22.2
Changes in Radiation Protection Standards for the Radiation Worker

Recommended Rate ^a	Comments
0.2 R/day (or 1 R/week) ^b	Recommended as a tolerance exposure by ICRP in 1934 and continued in worldwide use until 1950
0.1 R/day (or 0.5 R/week) ^b	Recommended as a tolerance exposure by NCRP on March 17, 1934, and continued in use in U.S. until 1949
15 rem/year (or 0.3 rem/week)	Recommended as an MPD by NCRP on March 7, 1949, and by ICRP in July 1950; continued in use until 1956
5 rem/year (or 0.1 rem/week)	Recommended as MPD by ICRP in April 1956 and by NCRP on January 8, 1957
5 rem/year (50 mSV/year)	NCRP, 1993
2 rem/year (20 mSV/year)	ICRP, 1991 for a 5-year, 5 rem (50 mSV) in one year

^a The values are in addition to medical and background exposure.

^b Based on a 5-day work week.

TABLE 22.3
Estimated RBE Values as a Function of Radiation Type and Energy

Type and Energy Range of Radiation	Radiation Weighting Factor (WR) or RBE
X-ray, γ -ray, β -particles, electron, all energies	1
Neutrons	
<10 keV	5
10–100 keV	10
100 keV-MeV	20
2–20 MeV	10
>20 MeV	5
α particles and other heavy particles	20

Data are summarized from ICRP Publ. No. 60, Pergamon Press, New York, 1991.

$$\text{Equivalent dose} = \text{Absorbed dose} \times \text{Radiation weighting factor (RBE)}$$

B. TISSUE WEIGHTING FACTORS (WT)

WT is the relative contribution of each tissue or organ to the total damage following whole-body exposure. The values of WT for some organs are listed in Table 22.4. For all organs,

$$\text{Effective dose} = \text{Absorbed dose} \times \text{WR} \times \text{WT}.$$

All dose limits in Tables 22.5–22.7 include the sum of internal and external exposure. The NCRP considers an annual effective dose of 1 mrem (0.01 mSV) to be a negligible individual dose.

These MPD values exclude contributions from medical exposure and natural background radiation. Because of the difference in the RBE of various types of radiation, the MPD is expressed in rems (SV), which takes into account the RBE in estimating the MPD value.

III. MAXIMUM PERMISSIBLE BODY BURDEN

The hazards of radioactive nuclides deposited internally may be estimated by reference to the maximum permissible body burden for the nuclide. The *body burden* of a particular

TABLE 22.4
Estimated Tissue Weighting Factors for Some Organs

Organ	Tissue Weighting Factor (WT)
Gonads	0.20
Bone marrow (red)	0.12
Colon, lung, stomach	0.12
Liver, bladder, breast, esophagus, thyroid	0.05
Skin, bone surface	0.01
Remainder	0.05

Data are summarized from ICRP Publ. No. 60, Pergamon Press, New York, 1991.

TABLE 22.5
Recommendations on MPD to Radiation Workers

Type of Effect	ICRP	NCRP
Annual effective dose for stochastic effect (cancer and heritable effect)	2 rem (20 mSV) average over 5 years, not exceeding 50 mSV in one year	5 rem (50 mSV)
Cumulative effective dose for stochastic effect		1 rem × age (10 mSV × age in years)
Annual effective dose for tissue (non-stochastic effect)		
Lens of eye		15 rem (150 mSV)
All other organs		50 rem (500 mSV)

Data are summarized from ICRP Publ. No. 60, Pergamon Press, New York, 1991, and from NCRP No. 116, 1993. No occupation exposure should be permitted before 18 years of age.

radioactive nuclide in a particular individual is the amount of nuclide in microcuries that is present in the individual's body. The body burden is influenced by the rate of cellular uptake, elimination of the nuclide, and radioactive decay. The *maximum permissible body burden* is the body burden for a particular radioactive nuclide that results in an MPD to the whole body or to one or more organs in the body. The maximum permissible burden is computed with the assumption that the radioactive nuclide of interest is the only radioactive nuclide in the body. Some of the factors that must be considered in estimating the maximum permissible burden are (1) energy and type of radiation, (2) concentration of radioisotopes in the body, (3) chemical form, and (4) physical half-life of the radionuclides. The maximum permissible body burden for a radioactive nuclide of a bone-seeking element (e.g., strontium, calcium, radium, or plutonium) is the number of microcuries required to deliver to the bone a dose in rems equal to that provided by 0.1 μCi of ²²⁶Ra in equilibrium with its decay products.

Body burdens for radioactive nuclides other than bone seekers require the identification of *critical organs* for the nuclides. The selection of a critical organ or organs for a particular radioactive nuclide in a particular chemical form requires the evaluation of many factors, including (1) the relative concentration of the nuclide in different organs, (2) the relative sensitivity of different organs to radiation, (3) the relative importance of different organs to the health of the individual, and (4) the radiation dose to the organs delivered during intake and elimination of the nuclide. In most cases, the concentration of the nuclide in various organs is the dominant influence in the selection of a critical organ. If a radioactive nuclide is distributed fairly uniformly throughout the body, then the whole body may be selected as the critical organ. For a nuclide with the whole body as the critical organ, the maximum

TABLE 22.6
Recommendations on MPD to Individual Members of the Public

Type of Effect	ICRP	NCRP
Annual effective dose for stochastic effect		
Continuous or frequent		0.1 rem (1 mSV)
Infrequent exposure	0.1 rem (1 mSV)	0.5 rem (5 mSV)
Education and training exposure		0.1 rem (1 mSV)
Annual effective dose for tissue (non-stochastic effect)		
Lens of eye; skin; and extremities		5 rem (50 mSV)

Data are summarized from ICRP Publ. No. 60, Pergamon Press, New York, 1991, and from NCRP No. 116, 1993.

TABLE 22.7
Recommendation of MPD to Fetuses

Type of effect	ICRP	NCRP
Total effect		0.5 rem (5 mSV)
Dose for stochastic effect	0.2 rem (2 msv)	
One month effective dose		0.5 rem (5 mSV)

Data are summarized from ICRP Publ. No. 60, Pergamon Press, New York, 1991, and from NCRP No. 116, 1993.

permissible body burden for occupational exposure is the activity present continuously in the body that delivers a dose equivalent of 5 rem/year to the whole body. Radioactive nuclides (e.g., ^{35}S , $^{127\text{m}}\text{Te}$ in soluble form) that concentrate in the testes are assigned maximum permissible body burdens for a dose of 5 rem/year to the testes. Nuclides that concentrate in abdominal organs (e.g., liver, spleen, kidney, and GI tract) are given *limiting* body burdens, which provide 15 rem/year to these organs. If the critical organ for a radioactive nuclide is the thyroid or skin, then a dose of 30 rem/year is used to compute the maximum permissible body burden for the nuclide.

IV. MAXIMUM PERMISSIBLE CONCENTRATION

Restriction of the uptake of radioactive nuclides into the body may be achieved by controlling the concentration of radioactive nuclides in the air that people breathe and the water that they drink. Maximum permissible concentrations in air and water have been established for exposures of 40 hr/week. Concentrations in air and water less than the maximum permissible concentration for a particular radioactive nuclide should provide a body burden for the nuclide that is less than the maximum permissible body burden.

V. BASIC ASSUMPTIONS IN SETTING MPD

The basic assumptions of the ICRP in setting the occupational MPD values are as follows:

1. Ionizing radiation is both harmful and beneficial to man. When properly used, it can be a powerful tool in research and can be useful in medical diagnosis and therapy; however, radiation induces many types of radiation damage, including malignancy.
2. The objective and indeed the purpose of the health physicist is to enhance and maximize the benefits of ionizing radiation while striving to minimize its harmful effects. Thus, we have the concept of weighing the benefits against the risks and of permitting exposure to manmade sources of ionizing radiation only insofar as it can be expected to yield benefits that are greater than the hazards.
3. The MPD levels should be set so that in the light of present knowledge, (1) they carry a negligible probability of severe somatic or genetic injuries (for example, leukemia or genetic malformations that result from exposure to individuals at the MPD would be limited at an exceedingly small fraction of the exposed group), and (2) the effects ensuing more frequently are those of a minor nature that would not be considered unacceptable by exposed individuals and by the society of which they are a part. Such frequently occurring effects might be, for example, modifications in the formed ele-

ments of the blood or changes in bone density. Such effects could be detected only by very extensive studies of exposed individuals. Effects such as shortening of the life span, which may be proportional to the accumulated dose, would be so small that they would be hidden by normal biological variations, and perhaps could be detected only by extensive epidemiological studies.

Publications of the NCRP and ICRP indicate that, although the MPD values for the occupational worker and the dose limits for members of the population at large are considered to be reasonable radiation protection standards, they should not be considered as levels above which one is in serious danger or below which the probability of radiation damage (even serious radiation damage, such as leukemia) is zero. The risk of serious somatic damage to the individual at these exposure levels is very low.

VI. IS THE MPD SAFE?

Most radiobiologists agree that there is no threshold dose for radiation-induced damage. Therefore, the human population should not be exposed to unnecessary radiation doses. Some have suggested that the natural background radiation to which man has always been subjected may be used as a suitable reference standard in setting up the MPD value. However, the background radiation cannot be used as a basis for determining the MPD value. This is because no useful epidemiological study has ever been conducted to determine the effects of the natural background radiation dose on the human population. On the basis of linear hypothesis, it has been estimated that, in the world population, natural background radiation may cause 70,000 deaths per year from cancer, life-shortening, and first-generation deaths, and may introduce into the population about 300,000 deaths per year — most appearing in future generations.⁸ It is doubtful if a meaningful study could ever be made to determine the effect of background exposure on humans, because there are many other factors in the environment, in diets, and in lifestyle that might modify the effect of ionizing radiation.

Both the NCRP and ICRP have made prudent assumptions that there is no threshold dose of ionizing radiation below which the probability of radiation damage to man is zero. The assumption is made that there is more or less a linear relationship between the accumulated dose and its effect on man. Recent studies indicate that the linear hypothesis is a reasonable one, even down to very low doses and dose rates. The dose-response curves for most malignancies are linear, but some require threshold doses. The dose-response curves for genetic mutation require no threshold dose. MacMahon⁸ and many others have reported an increased incidence in leukemia of 30–50% among children whose mothers were exposed to 1–3 rads of diagnostic dose during pregnancy. Stewart and Kneale¹³ reported that the incidence of crude excess cancer risk increases linearly as a function of the number of diagnostic X-ray films during pregnancy. Finkel et al.³ reported that there is a gradual progression from no symptoms to symptoms that are mild, moderate, advanced, and, finally, malignancies, as the body burden of ²²⁶Ra is increased from very low to high levels. The risk of malignancy is not zero, even in the range of the maximum permissible body burden (0.1 µCi). Some investigators have doubts about the effect of a low dose of radiation on human beings. Some data indicate⁹ that the ratio of somatic to genetic damage after a given dose of radiation is 30–60 times greater than was originally thought; therefore, somatic damage must be given increased consideration in setting population dose limits. In addition, the identification of the two groups of individuals, one of which is more susceptible to radiation than the other, indicates that the current MPD values cannot be applied to all populations. However, it has been emphasized that radiation exposure should be kept as low as is reasonably achievable (ALARA). Currently, all radiation exposure limits are governed by the ALARA principle.

In response to a federal request, the National Academy of Sciences-National Research Council Advisory committee on the Biological Effects of Ionizing Radiation (the BEIR Committee) undertook an exhaustive, critical review of the scientific literature and of additional unpublished information in this field. The purpose was to report the state of science against which the adequacy of federal radiation guides may be weighed. The BEIR report; *The Effects on Population of Exposure to Low Levels of Ionizing Radiation*, was transmitted to the Radiation Office of the Environmental Protection Agency.

VII. EXPRESSION OF GENETIC RISK

Present estimate of genetic risk is expressed in four ways.

A. RISK RELATIVE TO NATURAL BACKGROUND RADIATION EXPOSURE

Exposure to manmade radiation below the level of background radiation will produce additional effects that are less in quantity and no different in kind from those which man has experienced and has been able to tolerate throughout history. Continuous exposure of the population to the background radiation of 3 rem/30-year generation increases the rate of mutation by 1–6% of spontaneous mutation.

B. RISK ESTIMATES FOR SPECIFIC GENETIC CONDITIONS

The expected effect of radiation can be compared with the current incidence of genetic effects by use of the concept of doubling dose (the dose required to produce a number of mutations equal to those that occur naturally). Based mainly on experimental studies in the mouse and *Drosophila*, and with some support from observations of human populations in Hiroshima and Nagasaki, the doubling dose for chronic radiation in man is estimated to be about 100 rem. It is calculated that the effect of 170 mrem/year (or 5 rem/30-year reproduction generation) would cause, in the first generation, between 100 and 1800 cases of serious, dominant, or X-linked diseases and defects per year (assuming 3.6 million births annually in the U.S.). This is an incidence of 0.05%. At equilibrium (approached after several generations) these numbers would be about five-fold larger. Added to these would be a smaller number caused by chromosomal defects and recessive diseases.

C. RISK RELATIVE TO CURRENT PREVALENCE OF SERIOUS DISABILITIES

In addition to those described in the previous section, "Risk Estimates for Specific Genetic Conditions" — which are caused by single-gene defects and chromosomal aberrations — there are congenital abnormalities and constitutional diseases that are partly genetic. It is estimated that the total incidence from all these, including those in the previous section, would be between 1,110 and 27,000/year at equilibrium (again, based on 3.6 million births). This would be about 0.75% at equilibrium or 0.1% in the first generation.

D. THE RISK IN TERMS OF OVERALL ILL HEALTH

The most tangible measure of total genetic damage is probably "ill health," which includes (but is not limited to) the above categories. It has been estimated that 5 rem per generation would eventually lead to an increase of 5% in the ill health of the population.

Until recently, it had been taken for granted that genetic risks from exposure of the population to ionizing radiation near background levels were of much greater importance than were somatic risks. However, this assumption may no longer be valid. It has been estimated that the exposure of the U.S. population to 170 mrem/year for 30 years could cause about 3000–15,000 cancer deaths annually, depending on the assumptions used in the calculations. The Committee considers the most likely estimate to be approximately 6000 cancer deaths

annually, an increase of about 2% in the spontaneous cancer death rate, which is an increase of about 0.3% in the overall death rate from all causes.

There is reason to expect that over the next few decades, the dose commitments for all manmade sources of radiation (except medical) should not exceed more than a few millirems average annual dose to the entire U.S. population. The present guides of 100 mrem/year grew out of an effort to balance societal needs against genetic risks. ICRP has estimated that overall risk from cancer and serious heritable effects is about 7/100 person/SV (700/10⁶ person/rem).

The current exposures from medical and dental uses can be reduced without impairing the benefits of radiation.

Excessive use of human chest X-ray surveys is often conducted in spite of the fact that the Public Health Service has indicated that such programs should not be given to all population groups,⁷ but only to those groups where the incidence of tuberculosis is high, and notwithstanding the observation of the World Health Organization:¹⁴ "Attempts have been made to use radiography for detecting lung cancer in an early stage. The results have been relatively poor, and little if any improvement in the prognosis has been achieved."

It appears that patients in the U.S. are exposed to diagnostic exposure much more than in other advanced countries. In 1964, it was estimated that the genetic significant dose (GSD) from medical exposure in the U.S. was 55 mrem/year; however, a survey in 1970 indicated (John Villforth indicated this at the midyear symposium of the Health Physics Society in 1970) that this GSD value rose from 55 to 95 mrem. The GSD value from medical exposure in other countries are as follows:

U.K.	14 mrem/year
France	58 mrem/year
Japan	39 mrem/year
Sweden	38 mrem/year
Switzerland	22 mrem/year

VIII. SUMMARY AND COMMENTS

The maximum permissible dose (MPD) has been based on genetic considerations. Since recent data indicate that certain types of somatic damage are more sensitive to radiation than genetic damage, somatic damage must be given increased consideration in setting the population dose limit. There is no dose of radiation that can be considered "safe" or "harmless." Therefore, continual efforts must be made to minimize the radiation exposure of the individual. Before determining any amount of radiation, we must show that benefits derived from such exposure far exceed the possible harm. The nuclear industry will expand rapidly in the future. Therefore, all efforts should be made to minimize the exposure of the individual or population. The extent to which these efforts are successful may well affect the growth of nuclear energy in the future.

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Chapter 23

RADIATION RESPONSE OF HUMAN TUMORS

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I. INTRODUCTION

The radiosensitivity of human tumors varies markedly. The radiosensitivity of a tumor is measured by the rate of tumor regression during irradiation, and the efficacy of radiation therapy is determined by a partial or complete remission of tumor. Those patients surviving 5 years or more are considered cured.

II. FACTORS AFFECTING THE RADIOSENSITIVITY

The factors that influence the radiosensitivity of human tumors are not adequately understood. Some factors that are known to influence the radiosensitivity of tumors are (1) organ origin, (2) oxygenation, (3) rate of proliferation, (4) extent of differentiation, and (5) position of cells in the cell cycle. However, in many cases the radiosensitivity of the tumors cannot be explained by any of the above factors; therefore, in such cases the endogenous cellular factors, such as alterations in oncogenes and/or cellular genes, may be important in determining the radiosensitivity of the tumor.

A. ORGAN ORIGIN

It is generally believed that tumors arising from radiosensitive organs are radiosensitive, whereas tumors arising from radioresistant organs are radioresistant.¹ However, there are many exceptions to this broad concept. For example, testicular tumors, such as seminomas, are radiosensitive teratocarcinomas; and choriocarcinomas are radioresistant. Similarly, rhabdomyosarcomas are radioresistant, but embryonal rhabdomyosarcomas are radiosensitive — although both tumors are derived from the same cell type. The differences in radiosensitivity of astrocytomas and medulloblastomas, which are derived from astrocytes and dividing neuroblasts, respectively, represent another exception to the organ-origin-based concept of tumor radiosensitivity. For example, medulloblastomas are more radiosensitive than astrocytomas.

The radiosensitivity of tumors derived from the same organ varies if they arise from two different cell types. Thus, an ovarian tumor may be radiosensitive or radioresistant, depending on the cells from which it arises.¹ For example, granulosa cell carcinomas (which are derived from more radiosensitive tissue granulosa) are sensitive to irradiation, whereas pseudomucinous tumors (which are derived from the epithelial lining of the capsule) are radioresistant.

The radiosensitivity of tumors also varies markedly for the same type of tumors, e.g., squamous cell cancers.

B. OXYGENATION

The aerobic cells are generally more radiosensitive than the hypoxic cells.² It is possible that some tumor cells may show radioresistance in spite of adequate oxygenation. This may be due to an alteration in receptor response to external ligand or alterations in the expression of certain oncogenes or cellular genes.

C. THE RATE OF PROLIFERATION

According to the law of Bergonié and Tribondeau, the higher the rate of proliferation, the greater the radiosensitivity. Many tumors do not follow the above principle of radiosensitivity. This may be due to changes in the intracellular molecular environment.

D. EXTENT OF DIFFERENTIATION

According to the law of Bergonié and Tribondeau, the degree of cellular differentiation is inversely related to the radiosensitivity of cells. The undifferentiated cells are more radiosensitive than the differentiated ones. Therefore, one might expect that anaplastic cancers are radiosensitive, whereas well-differentiated cancers are radioresistant. However, the efficacy of radiation therapy cannot be predicted on the basis of a histological grading of tumors with respect to cell differentiation. It has been shown³ that the 5-year survival rate in differentiated carcinomas of the cervix was 23% greater than in anaplastic cancer for all stages. A further study⁴ showed that the survival for stage I of carcinoma of the cervix was 86.7% for differentiated cancer and 88.5% for anaplastic cancer.

E. POSITION OF CELLS IN THE CELL CYCLE

Generally, on the criterion of reproductive death, mitotic cells are most radiosensitive. Therefore, the efficiency of radiation therapy may depend upon the proportion of cells that may be in mitosis during irradiation. Unfortunately, the cell-cycle parameters *in vivo* are difficult to measure. In addition, it is very difficult to achieve *in vivo* synchronization. Therefore, it is difficult to design a treatment protocol in which radiation therapy is delivered at an appropriate time when most of the cancer cells are in an optimal radiosensitive cell-cycle stage.

III. RADIOSENSITIVITY VS. RADIOCURABILITY

Radiosensitivity is sometimes used synonymously with *radiocurability*. However, it should be emphasized that the above parameters are two entirely different criteria of measuring radiation response of tumors. In the case of radiosensitivity, one measures the cell death and reduction in cell proliferation, which may result in the shrinkage of a tumor. In the case of radiocurability, one measures the 5-year survival rate. Although both parameters appear to be related, in many cases there may not be any link. For example, the tumor may show "considerable" shrinkage following radiation therapy, but may not be radiocurable because of the presence of radioresistant cells. Therefore, when comparing the radiation response of two types of tumors, one should not confuse radiosensitivity with radiocurability.

IV. RADIATION-INDUCED CHANGES IN TUMORS

A. BLOOD VESSELS OF TUMORS

Neoplasms consist of two parts: tumor cells and vasculoconnective tissue stroma. These newly formed blood vessels are different from normal vessels because their rapid growth causes an imperfect architecture. Many consist of irregular channels with patchy endothelium and, in places, are lined by tumor cells. Elastic tissue or smooth muscle is generally absent from the vessel walls. There are three basic patterns of vascularization: (1) peripheral vascularization, (2) central source of vascularization, and (3) peripheral vascularization with avascular epithelial tumor cores.¹

1. Peripheral Vascularization

As a cancer increases in size and invades surrounding tissues, the host vessels dilate, become tortuous, and develop a network of peripheral vascularization. As the tumor further increases in size, the extent of peripheral vascularization increases. The rapid increase in the size of a tumor must be associated with its ability to sustain a sufficiently rich vascular supply. As the tumor grows in size, central necrosis occurs. Although peripheral vascularization with avascular epithelial tumor cores exists, it is uncommon.¹

2. Central Source of Vascularization

Major branches from the host blood vessels supply the tumor. The pattern of vascular supply is that of a branching tree with large branches in the centers of tumor nodules, which grow by forming smaller branches, thus giving a nodular pattern. There is little necrosis, despite the large size of the tumors, because the vascular tree follows the infiltrating tumor edge.¹

3. Radiation Damage to Tumor Vessels

The damage of blood vessels may lead to hypoxia and tissue necrosis. The hypoxic cells are known to be resistant to X- or γ -irradiation. Therefore, for an effective regression of the

tumor, the vascular system must be preserved during the radiation therapy.¹ After radiation therapy, the inflammatory response due to the destruction of tumor cells leads to the *supervascularization effect* of regressing tumors, which, in turn, causes better circulation and better oxygenation of the tumor cells.¹ Therefore, such tumor cells may become more radiosensitive to subsequent irradiation.

Using experimental tumors, it has been shown⁵ that the functional intravascular volume significantly decreased during the period of 1–11 days after a single dose of 2000 rads. The increase in extravasation of plasma protein for a brief period soon after irradiation could be due to the formation or release of endogenous vasoactive mediator(s) rather than structural damage to blood vessels. The subsequent decrease in extravasation of plasma protein appears to be a mere reflection of decreased functional vascularity and is not caused by decreased vascular permeability. Clearance of interstitially deposited ¹³³Xe (xenon) increased significantly 6 days after irradiation. It has been suggested that the irradiation-induced fragmentation and occlusion of capillary architectures could result in the formation of short shunts. Under such circumstances, the velocity of blood flow and the clearance of ¹³³Xe would be rapid in the tumor as a whole, although the blood supply to certain parts of the tumor may be sparse.¹

B. RELATIVE RESPONSE OF TUMOR CELLS *IN VIVO* AND *IN VITRO*

There is some evidence that cells *in vivo* can accumulate more sublethal damage than cells *in vitro*. The majority of cells grown *in vitro* maintain a D_q value below 200 rads; however, the D_q value of tumor cells *in vivo* is about 300–800 rads. These results suggest that extrapolation of *in vitro* results may considerably underestimate the effective dose required for tumor regression *in vivo*.⁶

1. Repair of Tumor Cell Damage

Although human tumor cells repair radiation damage, the kinetics and time of repair may vary from one tumor to another. In addition, a difference in the rate of repair of normal and malignant cells has not been adequately established.

Using three types of Syrian hamster tumors (reticulum-cell lymphosarcoma, fibrosarcoma, and malignant neurilemmoma) transplanted into the chick pouch, it has been shown⁷ that there was a transient delay in the growth or decrease in volume of all tumors at all levels of exposure (1000, 3000, or 6000 R). Recovery of primary tumor growth was inversely related to the exposure level. Frequency of tumor control increased and the incidence of metastases decreased with increasing exposure level. Based upon these parameters, fibrosarcoma is most sensitive to a single exposure, followed by malignant neurilemmoma and reticulum-cell lymphosarcoma. The reason for this differential radiosensitivity is unknown.

2. Cell Kinetics and Growth

The growth of a solid tumor is mainly determined by the relative rate of cell proliferation and cell death. There is a wide variation in the duration of the cell cycle in different tumors. Therefore, no significant correlation can yet be established between the type of tumor and the kinetics of the cell components.⁸ In a number of experimental solid tumors, the relationship between the volume increase and cell proliferation depends on tumor size.^{9,10} Results of these studies show that with increasing tumor volume, the fraction of proliferating cells may decrease and the cell cycle time may change. Furthermore, it has been shown⁹ that the fraction of proliferating cells and the cell cycle time are not constant parameters throughout a tumor.

3. Proliferation of Tumor Cells

The rate of proliferation after irradiation of a tumor is variable, depending upon the time after irradiation and radiation dose. Some animal experiments¹¹ have shown that the growth

rate of tumor cells after a single dose is faster than it was before irradiation, and that it decreases progressively during the regrowth of the tumor. Clinical observations on human tumors show similar variation.¹² The mechanism responsible for the increase of the growth rate after irradiation is unknown. It may be due either to the depopulation, which increases the availability of nutrients, or to a stimulation by the killed cell.¹³

V. POTENTIAL TUMOR DOUBLING TIME (T_{pot})

The T_{pot} is a measure of the rate of increase of cells capable of continued cell division.²³ The fraction of cells in the cell cycle at any given time is referred to as the *growth factor* (GF), and the fraction of dividing cells that become hypoxic is called the *cell loss factor*.

$$T_{pot} = \frac{\lambda T_s}{LI}$$

where T_s is the length of the DNA synthetic phase, and LI is the labeling index (the fraction of cells synthesizing DNA at any time). $\lambda = 0.67$ to 1.0 is a correction factor. Tumor with a short T_{pot} may repopulate if fractionation is extended for a long period of time.

VI. MODIFICATION OF RADIATION DAMAGE OF TUMOR CELLS

The study of the modification of radiation damage of tumor cells may help in developing new approaches to improve the efficacy of radiation therapy.

A. EFFECT OF DOSE RATE

Radiation-induced cell death is influenced by dose rate, but the dose rate difference must be at least a factor of 10. The higher the dose rate, the greater the damage. However, because both normal and tumor cells may show increased radiosensitivity as a function of dose rates of low-LET radiation, the high dose rates are rarely used to improve the efficacy of radiation therapy.

B. EFFECT OF FRACTIONATION

The schemes of fractionations used by most radiation therapists are based on empirical data and convenience. Most treatment regimes involve daily fractionation for 4–6 weeks (5 days/week). The fractionated doses of 200 rads/day (a total of 3000–4000 rads) are based on the tolerance of normal tissue. Larger single doses may be more effective in killing tumor cells, but they also may be more damaging to normal tissue. Therefore, they cannot be used for the routine radiation therapy practices. Fractionated doses are effective in radiation therapy for the following reasons: (1) increased oxygenation of hypoxic regions occurs due to formation of new blood capillaries following radiation-induced killing of tumor cells; (2) cells are reversibly blocked in the G₂ phase (division delay) following the first radiation exposure, and this causes an increased number of cells in radiosensitive phases of the cell cycle at a time when the next radiation dose is given; and (3) a differential rate of repair may exist between normal and tumor cells, normal cells exhibiting higher rates of repair.

Prolongation of treatment time during radiation has the advantage of sparing damage to normal tissue and allowing reoxygenation of tumor tissue. However, excessive prolongation of treatment time may allow surviving tumor cells to proliferate during therapy. Two separate strategies are used for multiple fractions per day:

1. Hyperfractionation
2. Accelerated fractionation

1. Hyperfractionation

The intent is to reduce late effects without significantly affecting tumor control. If the total doses are increased, the intent is to achieve the same late effects and better tumor control. Hyperfractionation is generally delivered two fractions per day; but the number of fractionations can be increased, and the size of dose per fractionation can be decreased. The overall treatment time remains the same as in standard fractionation schemes; however, the total dose can be increased because of reduced dose per fractionation.

2. Accelerated Fractionation

The intent is to reduce repopulation in rapidly proliferating tumors. The number of fractions and the total dose are the same as those used in standard fractionation. Two or more fractionated doses per day are delivered, and this leads to a marked reduction in the treatment time. The latter may be responsible for reducing the rate of tumor cell repopulation during radiation therapy. Because the total number of fractions, total dose, and dose per fraction are the same as with standard fractionation, no significant changes in the late effects are anticipated in comparison to those with standard fractionation. Accelerated fractionation schemes are found to be of some value only in those patients with fast-growing tumors.

C. EFFECT OF QUALITIES OF X-IRRADIATION

The availability of supervoltage therapy in the form of radiocobalt teletherapy and 1–6 MeV generating units has proved useful in radiation therapy, because skin reaction is either avoided or reduced and a better depth dose is achieved. However, with regard to control of the tumor, there is no biological advantage to supervoltage irradiation as compared to orthovoltage. In fact, most experimental studies suggest that its RBE is at least 10–15% less than that of orthovoltage radiation, and this is usually compensated for in the treatment.¹

D. EFFECT OF HIGH-LET RADIATION

High-LET radiations have greater RBE value; therefore, they may be more effective in killing tumor cells. In addition, the hypoxic cells, which are very resistant to low-LET radiation, are sensitive to high-LET radiation. Unfortunately, high-LET radiation would also produce more damage to normal tissue on the criteria of cell death and carcinogenesis (15 MeV neutron [RBE, 1.6; OER, 2], negative pions [RBE, 2; OER, 1.8], ^{252}Ca [RBE, 1.7–4.3; OER, 1.8]).

1. Neutron Irradiation

A transplanted mouse osteosarcoma is resistant to X-ray fractionated radiation therapy, which is probably due to the presence of hypoxic cells; although a small degree of oxygenation occurs during therapy. The RBE of fast neutrons (15 MeV) is 2.5; the OER is also about 2, which is slightly less than the OER of X-rays (2.4). Therefore, the use of fast neutrons in therapy of animal osteosarcoma does not offer any major advantage.¹⁴ In another study,¹⁵ neutron radiation, when given in fractionated doses, has a generally smaller OER than that of X-rays by a factor of 1.6–1.8. Relative RBE values¹⁶ of 15-MeV neutrons relative to 300-kV X-rays for damage to normal tissue are lower than for the tumor tissues. The results of fractionated irradiation of normal tissues indicate an increase in RBE value with decreasing dose per fraction. RBE values of normal tissue are also dependent on neutron energy. Comparison of RBE values of fast neutrons derived from published data¹¹ for experimental tumors and normal tissue indicate that overall gain factors, RBE tumor/RBE normal, range between 1.1 and 1.3.

2. Negative Pions Irradiation

The plateau portion of the negative pion beam is of very low LET, and the RBE is approximately 1.0. The RBE value at the peak region is relatively high. It has been estimated¹⁷ that the RBE of the negative pion peak for cell survival at high dose rates would be about 2, which is approximately the same as that of fast neutrons. At low dose rates, or with fractionation, the RBE value would be expected to be 4 or more.

3. OER of Negative Pions

The maximum OER value is obtained with low LET radiations that are found in plateau regions of the negative pions. The minimal value with very high LET is about 1.0. The OER also varies with dose rate¹⁷ (less so than does the RBE): the lower the dose rate, the lower the OER. The results of several experiments indicate that the OER for the peak portion of the negative pion curve is about 1.5–1.8, comparable to fast neutrons. Thus, the negative pions may have a distinct advantage over fast neutrons with respect to the ratio of *effective dose* to hypoxic tumor cells compared to that of the dose-limiting normal tissue, if the following conditions exist:

1. Normal tissue adjacent to tumor
2. Layer of normal tissue just below the skin surface
3. The stratum germinativum
4. Distal vital tissue beyond the tumors

Damage to the above tissues may be minimized by a negative pion beam.¹⁷

Clinical studies show that pions may be of very limited value in treating localized skin tumors such as melanomas, which are resistant to low-LET radiation.

E. HYPERBARIC RADIATION THERAPY

Oxygen increases the radiosensitivity of both normal and malignant cells. Therefore, hyperbaric radiation therapy has been used in the treatment of tumors containing hypoxic cells. During this procedure, the patients are enclosed in an oxygen chamber in which they are allowed to breathe at 3–4 atm during exposure. It has been reported that the hyperbaric oxygen therapy produced better survival than that in a normal atmosphere.¹⁸ A review of the available report¹⁸ with different types of tumors demonstrated significant improvement with oxygen only when 4–12 fractions were used. All studies revealed some benefits from oxygen in squamous cell carcinoma of the cervix and of the head and neck, regardless of the fractionation used. The benefits of oxygen are less striking and consistent in carcinoma of the urinary bladder, bronchus, and glioblastoma multiform because of the generally less-favorable long-range results and the high incidence of metastasis, especially in bronchiogenic carcinoma. The toxicity of oxygen limits the usefulness of hyperbaric therapy. The advantage of hyperbaric oxygen therapy was also observed in advanced cancer of the prostate and possibly in salivary gland malignancies, which, in general, are poorly responsive tumors.¹⁹

F. RADIOSENSITIZING AGENTS IN RADIATION THERAPY

Although there are numerous agents that increase the radiation response of tumor cells in culture and in experimental animal tumors, their value in improving the effectiveness of radiation therapy remains very limited. Many of these agents are extremely toxic in humans, and they do not provide any differential increase in radiosensitivity.

1. Alkylating Agents

The most commonly used alkylating agents are nitrogen mustard (HN_2), chlorambucil, cyclophosphamide (cytoxan), triethylenethiophosphoramide (thio TEPA), and triethylene

melamine (TEM). Of the alkylating agents, TEM has been of some value in combination with radiation in the treatment of retinoblastoma. The results of all alkylating agents on other tumors have been discouraging.

2. Actinomycin D

Actinomycin D inhibits the synthesis of messenger RNA and interferes with the repair of sublethal damage. Actinomycin D in combination with radiation has been useful in treating Wilm's tumor.

3. Amethopterin (Methotrexate) and Medroxyprogesterone

Methotrexate interferes with DNA synthesis, and thus it prevents cell replication by inactivating the folic acid coenzymes necessary for the synthesis of the DNA precursor, thymidylic acid. The clinical results with radiation and methotrexate have not been encouraging.

4. Hydroxyurea

Hydroxyurea kills cells of the S phase, which are generally radioresistant. This agent also partially synchronizes the cells in the G₁ phase of the cell cycle. Hydroxyurea, when combined with radiation therapy, has been of some value in the treatment of head and neck cancer.^{19,20}

5. Analogs of Purine and Pyrimidine

None of the analogs of purine or pyrimidine in combination with radiation has been useful in the treatment of human tumors.

a. Electronaffinic Compounds

Misonidazole is an excellent hypoxic cell radiosensitizer. Clinical results have been disappointing.

b. Hyperthermia

Hyperthermia (42.5–45°C) has been used in combination with radiation to treat human tumors. The overall clinical results have been disappointing, except for a transient local control of some tumors.

G. RADIOPROTECTIVE AGENTS IN RADIATION THERAPY

Radioprotective agents have not been useful in protecting normal tissue during radiation therapy of tumors. Most of the radioprotective agents are toxic in humans and provide no differential protection on normal tissue (a detailed discussion is in Chapter 5).

H. NEUTRON CAPTURE IN RADIATION THERAPY

This procedure involves the injection of boron-10 (¹⁰B) compounds and the capture of thermal neutrons (0.05 eV) by ¹⁰B, which produces α particles of about 11 μm in range by the following reaction: ¹⁰B (n, α) ⁷Li. Unfortunately, the currently available boron compounds do not accumulate in tumor tissue; therefore, the concept of neutron-capture therapy cannot be applied clinically until boron compounds are developed that selectively accumulate in tumor tissue.

I. EXTRACORPOREAL IRRADIATION THERAPY OF BLOOD NEOPLASM

The procedure of extracorporeal irradiation involves exposure of circulating blood outside the body. Extracorporeal irradiation of the blood has been used in chronic lymphocytic leukemia.²¹ Eleven patients with chronic lymphocytic leukemia were submitted daily to

extracorporeal irradiation of their blood. The irradiation was from a source of ^{137}Cs and ^{60}Co . The transit dose varied from 236 to 498 rads. The average daily exposure to irradiation was 4 hr. A marked reduction in the blood lymphocytic count was observed in most patients. This reduction was exponential and generally considerable. A reduction of volume of lymph nodes and spleen was seen in five patients, but it was less prominent than for circulating and megakaryopoiesis were observed in some cases. The secondary effects of this procedure were minor. This type of treatment may be useful to bring pregnancy to a completion in a woman with blood neoplasm.

J. RATIONALE FOR PRE- AND POSTOPERATIVE RADIATION THERAPY OF TUMORS

1. Preoperative Irradiation

Preoperative irradiation may be of some value in those patients in whom the tumor is in immediate proximity to, or infiltrating, a structure vital to life or function. A suitable reduction in the size and extent of the tumor or inactivation of the tumor extensions into the vital organs may render an inoperable patient operable.¹⁴ Preoperative irradiation may have the following advantages:²³

1. Increase the resectability of the tumor.
2. Reduce the potential of seeding of tumor cells during surgery.
3. Destroy microscopic foci of tumor, which may be beyond the surgical margins of resection.
4. Allow a smaller treatment field, because the operated area has not been contaminated with tumor cells.
5. Decrease complications that are associated with postoperative irradiation.

Preoperative irradiation may have the following disadvantages:²³

1. Inability to tailor irradiation to high-risk sites following the surgical procedures.
2. Delay in the primary treatment in those cases where surgery may be the choice of treatment.
3. Delay in wound healing.
4. Limitation of total radiation dose because of planned surgery.
5. Pathologic downstaging, which may influence selection of other adjuvant therapy.

2. Postoperative Irradiation

Postoperative irradiation may have the following advantages:²³

1. The extent of the tumor becomes known at the time of irradiation, and therefore, radiation treatment can be individually tailored.
2. The area of treatment can be better defined.
3. Operative wound will heal at a normal rate.
4. The potential of using unnecessary radiation doses in some patients is reduced.

Postoperative irradiation may have the following disadvantages:²³

1. Delivery of radiation treatment may be delayed because of poor wound healing or surgical complications.
2. Tumor may become poorly oxygenated following disruption of blood supply, and this may reduce tumor response to radiation.

K. RADIATION IN COMBINATION WITH CHEMOTHERAPY

Radiation, in combination with chemotherapy, is commonly used in the treatment of human cancers. This approach has not been effective in most adult tumors even in improving local control, except for cancer of the anal regions. This method has been useful in some pediatric tumors. Among the survivors of this treatment, the increased risk of second malignancies and nonmalignant diseases exists.

VII. SUMMARY AND COMMENTS

The radiosensitivity of human tumors markedly varies from one type to another and even among the same type of tumor. The radiosensitivity of a particular type of tumor in the same individual varies as a function of time. The factors that are known to influence the radiosensitivity of tumors are organ origin, oxygenation, rate of proliferation, extent of differentiation, and position of cells in the cell cycle. However, in the case of many tumors, radiosensitivity cannot be explained by any of the factors described above. There may be some endogenous cellular factors that also affect the radiation response of tumor cells. The isolation of a clone of varying radiosensitivity may provide some useful tool in the study of the radiosensitivity of tumors.

The regression of tumors following irradiation is due to both cell death and damage to the vascular system. The damage of blood vessels may lead to hypoxia and tissue necrosis. The hypoxic cells may escape radiation-induced reproductive death and, thus, may interfere with the effectiveness of radiation therapy. Therefore, for an effective regression of the tumor, the vascular system must be preserved.

There is some evidence to indicate that cells *in vivo* can accumulate more sublethal damage than cells *in vitro*. Cells *in vivo*, therefore, require a much higher dose than those *in vitro* to achieve the same amount of cell death. Tumor cells also repair, but kinetics and time of repair may differ from one tumor to another and from normal tissue. The quantitative difference in the rate of repair of normal and malignant cells has not been established as yet.

The radiation response of tumors can be modified by the dose rate, fractionations, LET, and radiosensitizing and radioprotective agents. The dose rate used in the clinical range does not significantly influence the tumor response. In general, the higher the dose rate, the greater the damage. At a lower dose rate (5 rads/min), cells repair. In tumor therapy, fractionation is more effective than a single dose, because greater amounts of radiation can be delivered, and because reoxygenation of the tumor cells occurs between fractions. In those tumors where the presence of hypoxic cells become the limiting factor in the control of tumor growth, the use of high-LET radiation may be useful for when (1) the RBE is higher than that of X-rays, and (2) the OER is lower than that of X-rays. Among high-LET radiations, fast neutrons and negative pions appear to be most effective. Some important radiosensitizing agents that have been of some value in the treatment of tumors are triethylene melamine (TEM) (retinoblastoma), actinomycin D (Wilm's tumor), methotrexate (squamous cell carcinoma of head and neck), hydroxyurea (head and neck cancers), 6-mercaptopurine (acute and chronic myelogenous leukemia), and 5-fluorouracil (carcinoma of gastrointestinal tract). The efficiency of neutron capture therapy in human tumors has not been encouraging.

Extracorporeal irradiation therapy also remains of limited value. This procedure may be useful in pregnant women who have blood neoplasms, because this may not produce any harmful effects on the baby and may keep the patient alive during the entire period of pregnancy. Radiation alone, or in combination with other chemotherapeutic agents and/or surgery, remains one of the best modes of controlling the growth of human tumors. The study of the modification of radiation damage of tumor cells on the one hand, and the mechanism

of radiosensitivity on the other hand, may help in developing new approaches to improve the efficacy of radiation therapy of human tumors.

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Chapter 24

RADIOISOTOPES IN BIOLOGY AND MEDICINE

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I. INTRODUCTION

Although the potential value of radioisotopes in biological and medical research was realized soon after their discovery, their usage remained very limited. This was due to the fact that the production of different types of radioisotopes was not technically possible. A major breakthrough in the production of radioactive materials came after the invention of the cyclotron by Lawrence of the University of California at Berkeley. Later, during World War II, Fermi and associates at the University of Chicago succeeded in achieving a chain reaction

with ^{235}U , which allowed the construction of a nuclear reactor. Today, most of the radioisotopes of biological and medical interest are produced either by a cyclotron or by a nuclear reactor. At present, radioisotopes are widely used in biological and medical research. The main references are listed at the end of this chapter.¹⁻⁷

II. GENERAL PRINCIPLES

To use radioisotopes most effectively and safely, one must know the following:

1. Physical properties of radioisotopes
2. Procedures of safe handling and safe disposal
3. Techniques of assaying radioactivity

A. PHYSICAL PROPERTIES

Before selecting a radioisotope for use in biological or medical research, one must be fully aware of the physical properties of radioisotopes, such as physical half-life, energy, type of radiation, and chemical form. The detailed physical properties of radioisotopes have been described in Chapter 3.

B. SAFE HANDLING AND DISPOSAL

Because radiation is an agent that can be beneficial as well as hazardous, it is necessary that the procedures involved in safe handling and safe disposal of radioactive materials be understood by users.

1. Procedures with β -Emitters

β particles have a relatively short range in tissues and, therefore, never constitute an external hazard; however, the high-energy β particles, when interacting with matters of high atomic number, produce bremsstrahlung radiation (X-rays). The energy of X-rays depends upon the atomic number of the absorber. For example, the 3-mm glass that stops the ^{32}P particles ($E_{\max} = 1.7$ MeV) serves as a source of X-rays whose maximum energy is over 1 MeV. Although only 1% of the total energy emerges in this way, a large quantity of such emitters, when shielded by only high-atomic-number materials, could pose an external radiation hazard. Therefore, high-energy β -emitting radioisotopes should be stored in a lead container that has an internal lining of a low-atomic-number material, such as aluminum. Aluminum stops the β particles and greatly reduces the bremsstrahlung. One of the greatest hazards with β -emitters is the handling of uncovered vessels containing the materials. In an open solution of ^{32}P with a concentration of 1 mCi/ml, the dose rate at the surface is about 13 rads/min. This will not be appreciably reduced by attenuation in a few centimeters of air, nor will there be much reduction — by the inverse square law — from a source of this kind. Obviously, a hand or face over such an open container may receive a considerable dose of radiation in a short time.

2. Procedures with γ -Ray Emitters

γ -Rays are much more penetrating than particles and require greater care to avoid excessive dose. Two methods are available for reducing intensity.

1. *Increasing the distance between radiation source and recipient:* The intensity of the radiation is inversely proportional to the square of the distance from the point source. If the source is not a point, the actual falling off of the intensity with distance is slower.

2. *Interposing a barrier to absorb radiation:* Lead is usually the best absorber of X- and γ -rays. The thickness of lead needed to reduce the intensity of radiation to a safe level depends upon the quantity of radioisotopes, the distance to the person, the duration of expected exposure, and the exposure rate.

Although the body of the person handling radioactive materials is protected by a lead shield, the hands and face may not be protected while the manipulations are carried out. Therefore, care must be taken to keep containers closed with a lid except during the actual pipetting or diluting. Pipetting by mouth should never be done, and pipettes or other instruments should be tilted so that the hand does not come over the open container. Forceps and other tools should have adequately long handles, but not so long as to cause awkwardness. Many special tools for the handling of radioactive materials are available commercially.

3. Transportation

When radioactive material is to be transported from one part of the hospital to another, the thickness of the portable lead container will depend on the amount and kind of isotope and the time in transit. Very heavy containers for large doses of radioactive gold or iodine should be mounted on a wheeled cart or table.

4. Administration of Radioactive Materials to Patients

In administering radioactive materials to patients by mouth, different procedures may be used depending on the quantity of the isotope. Tracer and small therapy doses are available from commercial suppliers in capsule form; the patient handles the capsule and swallows it with an adequate amount of water. When the tracer dose is in solution, it is usually poured into a paper cup, and the patient drinks it. For a therapy dose, the bottle is not taken out of its shield. The patient drinks the active solution through a beverage straw. Water is then poured into the bottle and drunk through the same straw, and the latter process is repeated to be sure that no radioactive residue remains. All used cups, straws, and wipers of any kind are taken back to the isotope laboratory for proper disposal.

The patient who receives radioactive material now becomes a potential source of radiation and must be treated accordingly. No tracer or test dose will make a patient radioactive enough to demand any specific precaution. However, radioactive vomitus or excreta can cause contamination and should be treated according to the prescribed method of disposing of radioactive materials. For large doses of radioactive materials, more definite precautions are necessary, and it has been recommended that patients receiving more than 30 mCi of ^{131}I be hospitalized. In the case of a large dose of ^{131}I for the treatment of thyroid cancer, the patients will excrete a considerable part of the isotope by way of the kidneys during the first 24 hr. It is desirable to save the urine in order to check excretion and retention. In hot weather, an individual who perspires profusely may eliminate an appreciable amount of ^{131}I by this route, and the bed clothes should be checked for possible contamination.

A patient with 100 mCi of ^{198}Au in the abdominal cavity emits radiation at the rate of 234 R/hr at 1 cm (point source). At 40 cm, a survey-type measuring instrument indicates about 100 mR/hr from such a patient immediately after treatment; the dose rate decreases with decay of the isotope. Personnel caring for these patients can carry out all indicated procedures for each patient's care, but should not spend more time near patients than is absolutely necessary.

5. Handling of Bodies Containing Radioactive Isotopes

It will occasionally happen that a patient requires emergency surgery shortly after receiving a therapeutic dose of radioactive isotope; or the patient may die, in which case an autopsy may be desired or the body will be embalmed. The handling of such bodies may pose problems of

radiation exposure for the surgeon, the pathologist, or the embalmer. This subject is treated in detail in the National Bureau of Standards Handbook 65, *Safe Handling of Bodies Containing Radioactive Isotopes*. In brief, a patient who does not have more than 5 mCi of radioactive material does not constitute a hazard for any of these procedures. If surgery or autopsy is to be done on the individual at the time when the isotope content is greater than this, the radiation safety officer should supervise the handling and disposal procedures.

The general safety routines are the following:

1. Keep laboratory and equipment clean at all times. Do not allow waste or contaminated materials to accumulate.
2. Wear rubber gloves and a laboratory coat for all operations in the "hot" laboratory.
3. Cover all trays and other work surfaces with disposable, absorbent paper, and handle radioactive materials on them.
4. Make sure that all containers of radioactive materials are properly labeled at all times, both with a statement of the kind and quantity of isotope and with a suitable radioactivity label, and keep all radioactive solutions covered.
5. Never pipette a solution by mouth, and never allow eating or drinking in the hot laboratory.
6. Have available a paper sack garbage can for immediate disposal of all contaminated waste, including paper wipes.
7. Try all new procedures with dummy runs not involving radioactive materials.
8. Monitor all work areas regularly, and employ standard personnel monitoring with either film badges or a monitor ionization chamber.
9. Give immediate attention to cleaning up any contamination.

6. Disposal of Radioactive Waste and Removal of Contamination

There are two general methods of handling the material; these may be described as dispersion and concentration. Dispersion is accomplished by mixing the radioactive material with so much diluting substance (water, air, or other substances) that constant intake of the diluted mixture will not result in an accumulation of a maximum permissible dose. Concentration is accomplished by reducing the volume as much as possible, and it is the necessary first step for burial or sea disposal.

a. In Sewage

Any soluble preparation of a short-lived isotope is generally disposed of by this route because of its convenience. The quantity that may be discharged at any one time depends on the isotope and on the average water outflow from the institution. The permissible levels for various isotopes in drinking water are given in the National Bureau of Standards Handbook 52. The problem is carefully analyzed for ^{131}I and ^{32}P in the National Bureau of Standards Handbook 49.

b. Stable Isotope Dilution

To any preparation of a radioactive isotope, enough "carrier", or stable isotope of the same element, can be added. This procedure limits the body burden of the radioactive isotope. If the person ingests the mixture with the small quantities of long-lived radioisotopes, this is a desirable procedure.

c. Incineration

Solid wastes in the hospital are usually disposed of in the institution incinerator. Such a disposal procedure is discussed in the National Bureau of Standards Handbook 65. The major objective of this procedure is to keep air contamination at a safe level. The materials to be

incinerated generally include paper wipes and tray liners at very low activity; therefore, they are permissible. However, if the wastes are highly radioactive, it may be advisable to store these materials in shielded containers for decay to suitable low levels. Animal carcasses and dry residues from chemical procedures may also be incinerated.

d. Soil Burial and Sea Disposal

These methods are suitable only for relatively large amounts of long-lived radioactive materials.

7. Contamination and Decontamination

Where procedures with radioactive materials are carried out in accordance with the rules suggested in the previous section, there is little likelihood of serious personnel contamination. Apparatus and the floor may be contaminated by an occasional accidental spill, or a patient who had a therapeutic dose of radioactive material may vomit.

These events may present major problems in the hospital. The entire subject is dealt with in the National Bureau of Standards Handbook 48, *Control and Removal of Radioactive Contamination in Laboratories*; familiarity with this handbook should be mandatory for all isotope users. Certain general rules for decontamination are:

1. Drop towel or absorbent material on spill.
2. Remove contaminated clothing and put it on a large paper for future check.
3. Scrub hands well with soap or detergent, but do not scratch skin surface.
4. Put on fresh rubber gloves.
5. If the spill is on the floor or table, take up as much as possible with blotters or absorbent paper. Use forceps to hold the solid radioactive waste. Place it immediately into the radioactive waste container. Clean further with a damp cloth and detergent; prevent spreading the contamination: avoid sloshing water.
6. Monitor contaminated material to determine whether clothing may go to the laundry and mopping materials to the incinerator, or whether they must be stored to allow decay of the radioisotope.

C. ASSAYING TECHNIQUES

1. Radiation Detectors

a. Ionization Chamber

There are several types of detectors. The simplest one is called an ionization chamber. At a saturation voltage, it measures the total number of ion pairs produced in the enclosed air space by radiation.

b. Proportional Counter

This counter measures current pulse, which is proportional to the primary ionization. When the applied voltage is increased beyond the saturation rate, primary electrons acquire enough kinetic energy to produce secondary and tertiary ion pairs. Therefore, the current pulse is greatly amplified. Because the pulse height or intensity is proportional to the ionization, the pulses can be sorted out in the electrical measuring device; therefore, β and α particles can be observed separately and independently of each other.

c. Geiger-Mueller Counter

This counter measures the ionization intensity produced by radiation. It requires a voltage higher than the proportional counter and cannot distinguish the pulses released by α -, β -, or γ -radiation.

d. Scintillation Counter

This measures the highly penetrating γ -radiation. Certain organic and inorganic crystals emit visible light when an ionizing radiation interacts with them. The intensity of light is essentially proportional to the energy of the incident radiation. The number of pulses is a measure of the frequency with which radiation bursts impinge on the scintillation crystals and, therefore, is a measure of the disintegration rate of a radioactive isotope.

e. Photographic Emulsion

The blackening of a photographic plate by X-rays is a well-known observation. The observed density of the film is a measure of the total radiation absorbed dose.

III. RADIOISOTOPES IN BIOLOGICAL RESEARCH

Radioisotopes are a powerful tool in the study of biochemical and physiological processes of the body. The fate of a given substance is followed within the body more accurately and more easily if the molecules are radioactive. The use of radioisotopes has greatly increased our understanding of the metabolism of nucleic acids, proteins, carbohydrates, and lipids. The mechanisms of action of hormones, drugs, and radiation have been extensively studied using a radioactive compound as a tracer. Several techniques are used for the investigation of biological problems; the two most important are (1) the autoradiographic technique, and (2) the biochemical technique.

A. TECHNIQUES

1. Autoradiographic Technique

This technique allows one to localize the site of macromolecular synthesis. It also permits one to follow the migration and transport of synthesized macromolecules from one site to another within the cell. No other technique is as easy and accurate for the above study as the autoradiographic technique. Using this technique, one can measure the different rates of macromolecular synthesis in a mixed-cell population. Let us take a specific example. If one is interested in finding the location of DNA synthesis within the cells of the small intestine, ^3H -thymidine (a specific precursor of DNA) is used for this purpose. A β -emitter of lower energy is most suitable for the autoradiographic technique. ^3H -thymidine (0.5 $\mu\text{Ci/g}$) is injected i.p. into the animal, and the sample of small intestine is fixed in 10% neutral formaldehyde. A paraffin section 2 mm thick is prepared and then dipped into a photographic liquid emulsion (Kodak NTB2, 1:1 in water). The slides are air-dried and kept in a light-tight black box containing calcium hydroxide to absorb the moisture. The slides are kept in the refrigerator for 1 or 2 weeks and then are developed like photographs. How long one should expose the emulsion-coated slides depends upon the concentration of radioisotopes and the metabolism of the molecules to be studied. After developing the slides, they are then stained with hematoxylin and eosin. β -radiation of ^3H inactivates the silver grains of the photographic emulsion, which appear black after development. The silver grains are primarily located in the nuclei of those cells that are synthesizing DNA. One can count the grains and estimate the rate of DNA synthesis.

a. Autoradiographs of Soluble Materials

If a radioactive precursor does not incorporate into insoluble products such as nucleic acid or protein, the radioactive tracer is washed away during the procedure of tissue sectioning. Therefore, for the autoradiographs of soluble materials, the tissue sections must be cut in a frozen state and then dried in a vacuum. The slides are then treated according to the usual procedure.

b. Autoradiographs and the Electron Microscope

Autoradiographs can also be prepared for an electron microscopic study in which one can localize the site of macromolecule synthesis on a subcellular level. Because the efficiency of this technique is poor, it is necessary to use a radioactive precursor of very high specific activity and in high concentration.

The radioisotopic technique has contributed greatly in the study of cell kinetics of both malignant and normal cells. However, in certain studies it is essential that the autoradiographic technique be combined with the biochemical one to obtain a better understanding of the molecular event occurring within the cells.

2. Biochemical Technique

Radioisotopes are used in the study of the metabolism of nucleic acids, proteins, carbohydrates, and lipids. The intermediary products of the above compounds can be separated, identified, and easily quantified by labeling the known position of an element or compound. In addition, one can identify the role of the labeled substance in the biosynthesis or degradation of a molecule.

IV. NEUTRON ACTIVATION ANALYSIS

This technique has been used extensively for the analysis of trace metal concentrations in various organs following X-irradiation, drug, or hormone treatment. The basic principle of neutron activation analysis is simple. The trace metals under investigation are made radioactive by bombarding the dry samples of liver, plasma, or spinal fluid with slow neutrons. During neutron irradiation, the stable trace metals, together with other elements, capture neutrons and become radioactive. By using a radiation detector, the amount of trace metal can be quantified. However, in organ samples, other elements (such as sodium, potassium, and chlorine, which have very high cross-sections for neutron capture) also become radioactive; this makes it impossible to quantify the radioactivity of a trace metal by single-channel analysis. Two techniques have been used to overcome the above difficulty.

1. *Use of multichannel analyzer:* The multichannel analyzer separates the photopeak of radioisotopes of different energy. By stripping off the energy contribution from other radioisotopes with the help of a computer, one can get a single photopeak of the radioactive trace metal under investigation.
2. *Chemical separation of activated trace metals:* Activated samples are digested in acid and mixed with a nonradioactive trace metal; the radioactive trace metal is precipitated chemically and then counted by a single-channel analyzer.

V. ^{131}I IN THYROID PHYSIOLOGY

Thyroid takes up iodine in the form of inorganic iodide (Na^{131}I). A major portion of injected iodine is accumulated in the thyroid and is used up in the formation of thyroxine hormone. Iodine is also concentrated in the saliva and in the gastric secretion, but has no physiological importance. Excretion of iodine is almost entirely via the urine, and only a small amount is lost in the stool. During lactation, iodine is secreted with the breast milk, and up to 4–5% of the tracer can be found in the milk after 24 hr. By the study of the uptake of ^{131}I , it is possible to study the physiological status of the thyroid. Most of the ^{131}I uptake studies have been done 24 hr after the administration of radioactive iodine. A thyroid uptake of over 55% is suggestive of hyperthyroidism; values of less than 20% are indicative of hypothyroidism. The normal

values in many subjects overlap with the hypo- or hyperthyroidism. Therefore, it is not advisable to rely completely on the thyroid ^{131}I uptake study for the physiological status of the thyroid.

VI. HEMATOLOGIC STUDIES

The following aspects can easily be studied by using the radioisotopic technique:

1. Determination of circulating red cell mass using ^{51}Cr in the form of sodium chromate.
2. Determination of total circulating plasma volume using human serum albumin labeled with ^{131}I .
3. Measurement of the total circulating blood volume.
4. Determination of red cell survival time using ^{51}Cr , ^{32}P , or ^{59}Fe .
5. Determination of rate of blood flow between two points using ^{24}Na , ^{42}K , or serum albumin tagged with ^{131}I .
6. Determination of the cardiac output using ^{24}Na .

VII. RENAL FUNCTION STUDY

The renal function is commonly evaluated with either $^{99\text{m}}\text{Tc}[\text{DTPA}]$ or $[^{131}\text{I} \text{ Hipp} ([^{131}\text{I}]ortho\text{-iodohippurate})$, or both. Recent experimental studies⁸ suggest that $^{99\text{m}}\text{Tc}$ -labeled N,N' -bis(mercaptoacetamide) ethylenediamine ($^{99\text{m}}\text{Tc}$ DADS) is useful. However, a recent study⁹ shows that the biological properties of $^{99\text{m}}\text{Tc}$ DADS are inferior to those of $[^{131}\text{I} \text{ Hipp}$. A new technetium chelating agent based on amide and mercaptide donor groups, N,N' -bis(mercaptoacetyl)-2,3-diaminopropanoate, has been synthesized as an analog of previously described N,N' -bis(mercaptoacetamide) ethylenediamine (DADS).¹⁰ The clinical results with this compound are not available at this time.

$^{99\text{m}}\text{Tc}$ -labeled diethylenetriaminepentaacetate (DTPA) is excreted from the kidney only through glomerular filtration. It is neither reabsorbed nor metabolized by the kidney. Therefore, it can be used to measure glomerular filtration rate (GFR). $[^{131}\text{I}]ortho\text{-iodohippurate}$ (OIH) and $^{99\text{m}}\text{Tc}$ -labeled mercaptoacetyltriglycine (MAG₃) are primarily secreted by the kidney tubules; therefore, they are used to measure an index of renal plasma flow. The advantages of $^{99\text{m}}\text{Tc}$ -DTPA are the ready availability of the $^{99\text{m}}\text{Tc}$ generator and a kit that is easy to prepare.¹⁵ $[^{131}\text{I}]OIH$ has the advantage of high excretion efficiency and cheapness; however, it has many limitations: (1) poor image quality, and (2) the radiation dose to the kidney is high. $[^{123}\text{I}]OIH$ is a better renal imaging agent than $[^{131}\text{I}]OIH$. Excellent images can be obtained even when renal function is poor. $^{99\text{m}}\text{Tc}$ -labeled MAG₃ can also be used in evaluating the function of the kidney. Radionuclide studies may be particularly useful under the following conditions:

1. Hypertension
2. Obstruction to outflow
3. Acute renal failure
4. Mass lesions in the kidney
5. Transplantation

A. RADIOTRACERS FOR THE BRAIN

$^{99\text{m}}\text{Tc}$ -labeled sodium pertechnetate or $^{99\text{m}}\text{Tc}$ -labeled glucoheptonate is commonly used for the study of a damaged blood-brain barrier (BBB). Cerebral blood volume is determined by

the use of ^{99m}Tc -labeled red blood cells or ^{99m}Tc -labeled serum albumin. ^{133}Xe , an inert and diffusible gas, is commonly used to determine cerebral blood flow.

VIII. LIVER FUNCTION STUDY

Two classes of ^{99m}Tc -labeled hepatobiliary agents are available. The initial series included ^{99m}Tc -labeled pyridoxylidene glutamate (Tc-PG) and ^{99m}Tc (Sn)-labeled *N*-pyridoxyl-5-methyltryptophan (Tc-PHMT).¹² The latter has shown rapid hepatobiliary kinetics with high specificity in animals. The other class of ^{99m}Tc -labeled hepatobiliary agents includes¹³ ^{99m}Tc -labeled *N*-(2,6-dimethylacetanilide) iminodiacetate (Tc-HIDA, Tc-dimethyl-IDA), *p*-isopropyl substitutions (Tc-*p*-isopropyl-IDA, Tc-PIPIDA), *p*-butyl substitutions (Tc-*p*-butyl-IDA, TcBIDA), 2,6-diisopropyl substitutions (Tc-diisopropyl-EDA, Tc-disofenin), and the 3-bromo-2,4,6-trimethyl substitution (Tc-mebrofenin, Tc-SQ-26, 962). The last Tc compound shows rapid hepatobiliary excretion kinetics and a high specificity.¹⁴

^{67}Ga -labeled citrate injection can detect over 90% of hepatocellular carcinoma; however, only 50% of metastases accumulate radioactive gallium. ^{67}Ga -labeled citrate or ^{111}In -oxine-labeled autologous leukocytes are often used to detect intra- or extrahepatic sepsis; vascular lesions in the liver can be detected by ^{99m}Tc -labeled human serum albumin or ^{99}Tc -labeled red blood cells. Nuclides of ^{111}In and ^{113m}In , when injected intravenously, attach to circulating transferrin, and images are taken by an appropriate pulse-height analyzer window set to exclude the ^{99m}Tc . Among radionuclide agents for imaging of the hepatobiliary system ^{99m}Tc -labeled iminodiacetic acid (IDA) is the best for routine clinical purposes. When assessment of biliary drainage is required over a period of several days, ^{99m}Tc with a half-life of 6 hr may not be adequate for this purpose. ^{97}Ru with a longer half-life may be adequate.

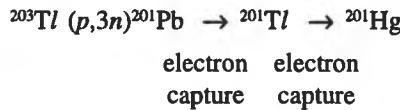
A. TUMOR IMAGING

Radionuclide brain imaging using ^{99m}Tc -labeled pertichnetate, diethylenetriamine pentaacetic acid (DPTA), or glucoheptonate commonly detects intracranial lesions with a sensitivity of 80–90%. ^{67}Ga and ^{99m}Tc -labeled pentavalent dimercaptosuccinic acid (VDMSC) have been used to detect squamous cell carcinoma of the head and neck. ^{67}Ga imaging provides 90% detection sensitivity in malignant lymphoma. ^{99m}Tc -VDMSC is used for detecting medullary carcinoma of the thyroid with a sensitivity of 65–88%. $[^{201}\text{Tl}]$ Thallous chloride can be used to detect all thyroid cancers including medullary carcinoma of the thyroid. ^{67}Ga has been widely used to evaluate the extent and location of lung tumors, and it provides sensitivity, ranging from 85 to 95%. However, adenocarcinoma of the lung takes up $[^{201}\text{Tl}]$ thallium chloride more readily than ^{67}Ga . Radio-labeled monoclonal antibodies are also being used to image lung tumors. Positron-emitting radio-pharmaceuticals are now used to study protein synthesis, glucose metabolism, and blood flow in lung tumors. ^{99m}Tc -labeled sulfur or tin colloid, or $[^{67}\text{Ga}]$ gallium citrate is commonly used to detect live cancer. ^{131}I - or ^{123}I -labeled metaiodobenzylguanidine (MIBG) is used to detect neuroectodermal tumors, such as neuroblastoma and pheochromocytoma.

B. CARDIOVASCULAR FUNCTION STUDY

Radionuclides have been used to study the heart and blood circulation. ^{99m}Tc -labeled human serum albumin or ^{99m}Tc -labeled red blood is used to obtain images of equilibrium-gated blood pool. ^{99m}Tc -labeled diethylenetriaminepenta acid (DTPA) or ^{99m}Tc -labeled sulfur colloid is used to evaluate heart functions, specifically if multiple studies are performed. Images of the myocardium can be obtained by injecting ^{99m}Tc -labeled macro aggregated albumin (30 μm diameter) through a catheter. The intravenous injection of monovalent cations ^{131}Cs , ^{81}Rb , and Tl causes distribution of these ions in proportion to cardiac output. Myocardial

ischemia can be identified in thallium scans by comparing the initial and redistribution images after exercise injection. ^{201}Tl is produced by cyclotron bombardment of stable ^{203}Tl with proton to produce ^{201}Pb , which decays to ^{201}Tl , which decays to stable ^{201}Hg .



Thallium imaging is used in three clinical areas: (1) detection of coronary artery disease, (2) post-therapy follow-up study, and (3) prognostication of the likelihood of a major ischemic event (sudden death infarction, or onset of unstable angina). The specificity of exercise thallium imaging for the detection of coronary artery disease is about 85%, compared with 82% for electrocardiography. $^{99\text{m}}\text{Tc}$ -labeled pyrophosphate ($^{99\text{m}}\text{Tc}$ -PYP) is commonly used to evaluate myocardial infarction.

Four new radiopharmaceutical generation systems have been developed and tested in human subjects for blood pool imaging: (1) $^{191}\text{Os}/^{191\text{m}}\text{Ir}$, (2) $^{195}\text{Hg}/^{195\text{m}}\text{Au}$, (3) $^{178}\text{W}/^{178}\text{Ta}$, and (4) $^{81}\text{Rb}/^{81\text{m}}\text{Kr}$. The combination of short daughter half-life and relatively high photon abundance makes these tracers useful for repeated assay of ventricular functions with a radiation dose of not more than 3–5 mrad/mCi.

C. POSITRON EMISSION TOMOGRAPHY (PET)

PET is a technique of radionuclide imaging which utilizes annihilation radiation from positron-emitting nuclides to record tomographic images. PET provides precise quantification that is not available with single-photon techniques. PET can measure fatty acid utilization and glycolysis in myocardium using $[^{11}\text{C}]$ palmitate and $[^{18}\text{F}]$ deoxyglucose.

IX. TREATMENT OF DISEASES

A. HYPERTHYROIDISM

^{131}I is used for the treatment of hyperthyroidism in humans. The dosage used varies, depending upon the disease and patient. On the average, about 5 mCi of ^{131}I is given in a single dose.

B. THYROID CANCER

^{131}I is used in the treatment of thyroid cancer. The therapeutic dose of ^{131}I is about 100 mCi. The total radiation dose to the blood per 100 mCi of ^{131}I averages about 52 ± 31 rads (cGy).

C. TREATMENT OF POLCYTHEMIA AND BLOOD NEOPLASMS

Polycythemia vera is a disease of unknown etiology characterized by an increase in the circulating red cell mass, which is caused by overproduction of these elements by the bone marrow and certain extramedullary sites — such as the liver and spleen. There is often an associated increase in the white cells of the myeloid series and the platelets. At present, it is generally accepted that ^{32}P is the most effective and most easily administered form of treatment for this disease, although some observers are concerned about a possible increase in the incidence of leukemia as a result of ^{32}P therapy. ^{32}P has been found to be useful in the treatment of chronic leukemia, but not in acute leukemia. Of course, the ^{32}P therapy of the chronic leukemias must be accompanied by proper systematic care and judicious use of transfusions and other affective agents, such as localized X-ray therapy, nitrogen mustard, antifolic acid preparations, urathene, and 6-mercaptopurine. ^{198}Au is used in carcinoma of the

prostate. Gamma radiation from ^{60}Co is widely used in the treatment of several types of malignant diseases.

The availability of radio-labeled monoclonal antibodies provides a new opportunity for the detection and treatment of human cancer. ^{131}I , ^{123}I and ^{111}In are commonly used radionuclides for labeling antibodies for treatment, whereas $^{99\text{m}}\text{Tc}$ is commonly used for detection purposes.¹⁶ In spite of extensive research and clinical trials, the results of radio-labeled antibodies for the treatment and detection of cancer has been disappointing. This is due to the fact that the tumor-specific antigen does not exist; therefore, radiolabeled nuclides accumulate both in normal and tumor tissues, thereby limiting the usefulness of this approach.

Bone metastases will eventually develop in 50% of patients with breast and prostate carcinoma. Management of bone pain is a significant problem in these patients. Phosphorus-32 (^{32}P) has been used as systemic therapy for the management of bone pain for over 40 years, but it causes hematologic depression. Recently, the bone-seeking radioactive compounds, such as strontium-90, samarium-153-ethylenediaminetetramethylene phosphoric acid, and rhenium-186-hydroxyethylidene diphosphonate, have been useful in the management of bone pain with acceptable hematological toxicity.¹⁷

X. SUMMARY AND COMMENTS

Radioisotopes are widely used in biology and medicine. To use radioisotopes most effectively and safely, one must know the physical properties of radioisotopes, procedures of safe handling and safe disposal, and techniques of assaying radioactivity. The use of radioisotopes in biological research has contributed significantly to our knowledge of intermediary metabolism, the synthesis of macromolecules, and cell kinetics. The radioisotope ^{133}I has been used in the study of thyroid physiology, and ^{59}Fe , ^{51}Cr , ^{32}P , ^{131}I , ^{24}Na , and ^{42}K have been used in the study of hematopoiesis. The liver, renal, and cardiac functions are evaluated by several new, primarily $^{99\text{m}}\text{Tc}$ compounds. Radioisotopes are also used in the treatment of certain diseases, such as hyperthyroidism with ^{131}I , thyroid cancer with ^{131}I , polycythemia with ^{32}P , and leukemia with ^{60}Co . Radioactive labeled antibodies are also used in the detection and treatment of human cancer with very limited clinical success.

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