# 5. MECHANISMS OF BIOLOGICAL EFFECTS

## 5.1 INTRODUCTION

Radiation ionizes cellular atoms and molecules; if immediate recombination does not occur, these can manifest as some type of molecular, cellular, or organic system alteration. An ionizing event can cause a variety of damage scenarios: (1) no damage if the ionized molecule reforms immediately; (2) repairable damage that causes no clinical effects if repaired; (3) repairable damage to DNA, which can be tumoriginic or carcinogenic if not repaired prior to cell division; (4) irreparable small-scale damage which causes cell death to a small population of cells that is insignificant and produces no clinical effects; and (5) irreparable large-scale damage that kills enough cells within an organ system to produce deterministic threshold effects such as cataracts and acute radiation syndrome

A very large radiation dose received in a short enough period of time to preclude significant repair can cause cellular walls to collapse and disrupt organ systems, producing deterministic effects such as acute radiation syndrome, cataracts, and teratogenesis (mental retardation, IQ reduction, microencephaly, stunted growth). These effects can be caused by acute exposure to sources of high intensity radiation, such as can be found in hospitals, government, and industry. Overexposure events which have caused such effects are not applicable to NPL site residual radioactive contamination. The discussion below largely relates to lower radiation doses and dose rates which can cause non-deterministic effects and which are more relatable to radiation exposure from NPL sites.

A number of direct and indirect radiation interaction pathways can produce damage to the DNA of irradiated cells. DNA damage occurs by indirect action (mediated through radiolytic products in water) or direct ionization. Cells depend on their DNA for coding information to make various classes of proteins that include enzymes, certain hormones, transport proteins, and structural proteins that support life. When the genetic information containing the “blueprint” for these substances is disrupted, cell homeostasis is disrupted, resulting in a wide-range of immediate and/or delayed toxicological effects. Direct and indirect ionization of DNA is ultimately responsible for the DNA alterations that adversely affect the structural and genetic integrity of the system. These alterations can be repaired, or can result in mutations in the genetic coding that can be passed on to daughter somatic cells or to progeny offspring

from reproductive cells. These alterations can result in the wide range of somatic and reproductive effects described in greater detail in Chapter 3.

The human body has nearly $10^{13}$ cells. Each somatic cell (cells other than sperm and eggs) contains 23 pairs of chromosomes. Each cell (except for red blood cells) contains a nucleus that houses these chromosomes. The total chromosomal content of a cell involves approximately $10^5$ genes in a specialized macromolecule of deoxyribonucleic acid (DNA). DNA is composed of alternating sugar and phosphate groups, with the sugar attached to 1 of 4 possible nucleotide bases (adenosine, cytosine, guanine, thymidine). These bases attach to each other in a specific pattern: adenosine:thymidine and cytosine:guanine. Genetic sequences of the bases are read in groups of three (called a triplet), with a possibility of 64 configurations or "words" in which to code information.

Specialized cell structures called ribosomes are the cellular organelles that actually synthesize the proteins (RNA transcription). RNA polymerases read the codes from specific areas of the DNA and transcribe the information into a mRNA copy of the DNA. At the ribosome, the processed mRNA is translated to produce proteins from amino acid units. When the genetic information containing the "blueprint" for these substances is disrupted, cell homeostasis is disrupted, with a wide range of possible non-carcinogenic and carcinogenic toxicological effects. These effects were described in some detail in Chapter 3. Radiation can disrupt the structure of the DNA (and other macromolecules), thereby disrupting normal cell and organ functions.

Direct macromolecule damage by radiation involves partial or complete energy transfer to one or more electrons on the molecule. Each electron that is given enough energy to overcome the attractive forces of the nucleus escapes from the DNA or other macromolecule and leaves it in the form of a charged ion; this process, called "ionization," is the source of the term "ionizing radiation" (see Chapter 2). Unlike non-ionizing radiation (such as microwaves and ultraviolet radiation), which has insufficient energy to eject molecular electrons, ionizing radiation deposits sufficient energy to remove electrons from atomic orbits and create molecular ion pairs along particle tracks.

Ionizing radiation can exert a number of adverse toxicological effects on many tissues in the body by ionizing and subsequently altering the DNA in the nucleus and other macromolecules of the irradiated

cell and possibly even the cytoplasm itself. This radiological toxicity occurs independently of, and in addition to, whatever chemical toxicological effects are produced by internally deposited radionuclides. Chapter 3 describes in some detail the biological effects of radiation in different organ systems in humans and laboratory animals and demonstrates that some systems or tissues are more sensitive to the effects of radiation than others. Chapter 3 also provides some general explanation for the presence of marked toxicity differences. DNA damage and cell wall destruction are the likely bases for lethality due to radiation; however, other molecules and cellular organelles may be damaged by radiation. These other molecular alterations may also result in adverse cellular activity and may be responsible for some of the biological responses observed after exposure to radiation. This chapter is an overview of the specific mechanisms that result in the non-carcinogenic and carcinogenic biological effects.

## 5.2 EVIDENCE OF THE EFFECTS ON DNA

Before any mechanism of action of ionizing radiation on DNA can be presented, it is necessary to demonstrate that DNA is indeed the critical molecule after exposure to radiation. Indirect evidence comes from studies which show that cells that divide frequently (undergo mitosis or meiosis in the case of spermatogonia) are the most sensitive to the effects of radiation. This phenomenon is described in Table 5-1. Conversely, structures that undergo less frequent mitotic cycles (myocytes, connective tissue, nervous tissue) are relatively more resistant to the effects of radiation. Early experiments, in which either the cytoplasm of the cell (not the nuclear material) or the nucleus only were irradiated with alpha radiation, demonstrated that the DNA is the most critical cellular component in radiation toxicology (Munro 1970). Those experiments showed that, although some minor effects could be induced after exposing the cytoplasm to alpha radiation, the nucleus (and the genome) were many times more sensitive to the effects of ionizing radiation. Recently developed research techniques, which allow precise irradiation of individual cell components with a predetermined number of alpha particles, have also concluded that cellular cytoplasm is less radiosensitive than DNA (Miller et al. 1999; Wu et al. 1999). These alterations are what ultimately gives rise to lethal or phenotypic genetic alterations of the DNA and may lead to the induction of many types of cancers in the irradiated individual (see Chapter 3 of this profile).

**Table 5-1. Relative Sensitivities of Major Organs and Tissues to the Effects of Ionizing Radiation**

| Radiosensitivity category | Organ system | General cell type affected | Frequency of mitosis |
|---|---|---|---|
| High | Lymphoreticular | Lymphocytes | Very frequent |
| | Hematological | Immature hematopoietic cells | |
| | Reproductive | Spermatogonia | |
| | | Ovarian follicular cells | |
| | Gastrointestinal | Intestinal epithelium | |
| | | Esophageal epithelium | Frequent |
| | | Gastric mucosa | |
| | Renal | Urinary bladder epithelium | |
| | Dermal | Epidermal epithelial cells | |
| | | Mucous membranes | |
| | Ocular | Epithelium of optic lens | |
| Medium | Circulatory system | Endothelium | |
| | Musculoskeletal | Growing bone and cartilaginous tissues | Moderately frequently |
| | Brain/CNS | Glial cells | |
| | Dermal | Glandular epithelium of the breast | |
| | Respiratory | Pulmonary epithelium, tracheobronchial epithelium | |
| | Renal | Renal epithelium | |
| | Hepatic | Hepatic epithelium | |
| | Endocrine | Pancreatic epithelium | |
| | | Thyroid epithelium | |
| | | Adrenal epithelium | |
| Low | Hematological | Mature hematopoietic cells (erythrocytes, neutrophils, eosinophils, basophils, macrophages) | Infrequently/rarely |
| | Musculoskeletal | Myocytes, osteocytes | |
| | | Mature connective tissues | |
| | | Mature bone and cartilage | |
| | Brain and peripheral nervous system | Ganglion cells | |

Other evidence exists to support the thesis that radiation's toxicological effects are intimately related to nuclear DNA damage. For example, when non-radioactive thymidine is incorporated into the DNA of a cell, no change in the cell's lifespan is encountered; however, when the same thymidine is labeled with radioactive tritium ($^{3}H$), which emits short-range beta particles (see Chapter 2), cell lethality dramatically increases. Coupled with the other indirect evidence, this suggests that the low-energy beta particles are

ionizing the nuclear DNA, resulting in increased incidences of cellular death. Additionally, work involving radioactive material in viruses and plants has shown a strong correlation between the chromosome volume of a cell with radiosensitivity—the larger the volume of chromosomal material, the greater the relative radiosensitivity of the cell. Radiosensitivity is relative to a specific biological end point. For instance, sperm have a high radiosensitivity with respect to mutation induction but a very low sensitivity with respect to cell killing. A direct correlation has been demonstrated between aberrant chromosome formation at the first cell division after irradiating hamster cells. These and many other studies provide strong evidence that exposure to radiation has detrimental effects on cellular DNA (Hall 1988).

## 5.3 INTERACTIONS OF IONIZING RADIATION WITH DNA

Chapter 2 provides an overview of the types of radiation and their ability to transfer energy when ionizing biological matrices. The interaction of radiation with all molecules (including DNA and other cellular components) may be classified as either direct or indirect interactions. Each produces damage by a specific pathway (or mechanism) that is described in more detail in this section.

Depending on the energy of alpha and beta particles when they are formed, the initial velocity of an alpha particle can be a few tenths the speed of light and that of a beta particle can approach the speed of light; however, these velocities reduce toward zero as they interact with the medium through which they pass, losing energy as they excite and ionize molecules along their paths. Since gamma rays are electomagnetic radiation, they travel at the speed of light even as their energy is transferred to the medium. As described above, a direct interaction occurs when an alpha particle, beta particle, or gamma ray hits and ionizes an atom or molecule. Both high and low LET radiation can directly ionize a molecule at the point of impact, producing two adjacent pieces which are chemically reactive. If the two pieces immediately recombine to reproduce the same original molecule, no damage results. Alternately, the pieces may drift apart, engaging neighboring atoms and molecules in any stabilizing chemical reactions that are thermodynamically feasible. Each chemical reaction produces a different molecular species. In the case of high LET radiation or high intensities of low LET radiation, the distance between ionizing events is short enough that the radiation can ionize adjacent molecules or even multiple bonds on the same molecule. For a large macromolecule such as DNA with its multistrand arrangement in chromosomes, these actions can damage the molecular structure in a number of ways. Radiation can remove large or small pieces of the molecules, and can open purine rings (leading to depurination) and break phosphodiester bonds. This action may result in the genetic effects listed in Tables 3-4 and 3-5.

Genotoxic effects are a major toxicological end point for exposure to ionizing radiation and are likely involved in the induction of cancer in humans. The data from Tables 3-4 and 3-5 demonstrate that the typical genotoxic effects associated with radiation exposure of genetic material primarily consist of deletions, mutations, chromosomal aberrations, and breaks, resulting in reciprocal translocations, sister chromatid exchanges, dominant lethal mutations, and sperm anomalies. When the cell enters a mitotic cycle, these damaged chromosomal units have an increased probability of failing to replicate properly due to structural damage unless chromosomal repair mechanisms repair the damage prior to entering mitosis. If the repair mechanisms fail to perfectly and seamlessly repair the damage to the chromosome, restoring it to its original preionized structure, or do not repair the damage at all, the chromosome may not replicate properly. This results in critical portions of that chromosome being deleted during the replication cycle, resulting in cell death (which equates to no damage at low doses) or genetic mutations in cell progeny. Table 5-1 shows that those cells that undergo rapid mitotic cycles (intestinal crypt cells, fetal cells, and other rapidly dividing cells) have less time for repair mechanisms to reverse the radiation damage to the nuclear DNA, making chromosomal anomalies more likely to be present during subsequent mitotic cycles and increasing the chances for cell death, genetic mutations, and abnormal cell functions in cell progeny. Ionizing radiation can affect other macromolecules in a similar fashion; these effects are discussed in Section 5.4.

Indirect interactions are molecular disruptions occurring at distances from the radiation's direct interaction site. Indirect interactions are mediated by radiation-produced chemical species (free radicals and oxidizers) with sufficient life-times and reactivity to diffuse away from the primary site and disrupt molecules with which they collide. Some of the radiation degradation (radiolysis) products of water, including the hydrogen and hydroxyl radicals produced by the reactions below, are recognized cytotoxins, and oxygen enhances these effects. Thus, oxygenated tissue is more radiosensitive than anoxic tissue. Water comprises approximately 60% of the total body mass of humans and laboratory animals, and 75–80% of the chemical composition of the living cell. When radiation interacts with water molecules surrounding DNA, the end products diffuse away and react with any DNA that is in their paths. Biological material that has a low water content, such as spores, exhibits a greater resistance to radiation effects.

Radiolysis of water

$$H_2O + IR \rightarrow e^- + H_2O^+$$
$$e^- + H_2O \rightarrow H_2O^- \rightarrow OH^- + H^\cdot$$
$$H_2O^+ \rightarrow H^+ + OH^\cdot$$

In the first reaction, radiation interacts with free cellular water to produce one free electron ($e^-$) and one ionized water molecule ($H_2O^+$), a reaction commonly known as radiolysis. This free electron is highly reactive and interacts with another un-ionized water molecule to produce a negatively charged and highly unstable water molecule. This molecule quickly decomposes to form the $OH^-$ ion and the $H^\bullet$ free radical; the $H^\bullet$ radical is reactive, but the $OH^-$ ion is more stable and can then diffuse out into the cellular fluid and interact with any number of macromolecules it encounters in its path, such as molecules of DNA. The remaining $H_2O^+$ molecule can also transform into a free and ionized hydrogen ion (potentially affecting intracellular or extracellular pH) and the hydroxyl radical. From these reactions, four products of radiolysis can occur after ionizing radiation interacts with a water molecule: $H^\bullet$, $OH^\bullet$, $H^+$, and $OH^-$.

Of the radiolysis products, 55% are either $H^\bullet$ or $OH^-$ and are the most important species biologically; however, they have lifetimes of approximately $10^{-11}$ seconds, which is long enough to produce damage to DNA and other macromolecules. These ionized particles will react with DNA, resulting in the addition of atoms or loss of atoms or pieces of the molecule; this will ultimately result in structural degradation, cross-linking, breakage of chemical bonds, and a host of other adverse effects. $H^\bullet$ or $OH^-$ may also interact with each other, to form an innocuous water molecule.

In the presence of water and oxygen, radiation can produce another set of reactions that have more potentially destructive capabilities within the cell. The radiolysis reaction, in the presence of molecular oxygen, results in the formation of three chemical entities: hydrogen peroxide ($H_2O_2$), hydroperoxy radicals ($HO_2^\bullet$), and hydroperoxy ions ($HO_2^-$). All have potent oxidizing potential and lifetimes of approximately $10^{-11}$ seconds. With an extended lifetime (when compared to the $10^{-11}$ second half-lives of $H^\bullet$, $OH^\bullet$, $H^+$, and $OH^-$), there is a greater diffusion length and potential for interacting with and inducing more damage to the DNA. Oxygen is, therefore, considered a radiosensitizing agent, associated with the production of relatively longer-lived and more potent by-products than in tissues containing less oxygen. The oxygen-water-ionizing radiation interactions have practical applications in clinical medicine. Radiotherapy is often used to treat large cancerous tumors in humans. Oxygen tension is lowest at the center of these large cancers, due to an inadequate blood supply to the cancer, compression from surrounding cells, or altered aerobic metabolism in these cancerous cells. Many of these masses may have liquified and necrotic centers as well. Low oxygen tension in these cancers may not result in the production of significant amounts of hydrogen peroxide and hydroperoxy ions/radicals to damage macromolecules within these abnormal cells and, therefore, may limit the efficacy of radiotherapy in

these patients. The use of oxygenating chemicals to offset this oxygen deficit and enhance the oxygen tension can make cancer tissue more radiosensitive than the surrounding healthy tissue.

Radiation frequently produces an important type of change to DNA at the molecular level by removing a base to form an apurinic or apyrimidinic site. The deletion or total destruction of DNA bases, destruction of deoxyribose residues, and deamination of cytosine or adenine are a few of the many ways radiation can alter the DNA at a molecular level. The attack by direct and indirect radiation action results in the degradation of bases and sugars, breakage of the hydrogen and sugar-phosphate bonds, and cross-linkages, all of which are deleterious to the structural integrity of the DNA macromolecule. Significant amounts of damage make the DNA unable to successfully replicate during mitosis and/or unusable for transcription into RNA. The magnitude of the damage is dose-dependant. A more in-depth discussion of the alterations at the DNA level by radiation, including a few of the known DNA repair mechanisms, is presented in BEIR V (1990).

DNA base damage is the most predominant type of DNA damage, followed (in decreasing order of incidence) by single-strand breaks, DNA-protein cross-linkages, and double-strand breaks. In base damage, thymidine appears to be the most radiosensitive base, followed by cytosine, adenine, and guanine. A 100-rad (1 Gy) dose of low LET radiation can produce 63–70 double-strand breaks per cell and 1,000 single-strand breaks (Cockerham et al. 1994). In addition, it was noted that there were 440 sites of multiple DNA strand lesions that are in close proximity to each other that interact in such a way to cause cell death (called Locally Multiple Damaged Sites [LMDS]). It would appear that simple single- or double-strand breakage is responsible for cell death; however, in cases of genotoxicity after chemical exposure, single-strand breakages have numbered into the hundreds of thousands, suggesting that the relatively low number of single-strand breaks after exposure to radiation is not likely to be the primary cause of cell toxicity, probably because of the presence of repair systems. Double-strand breaks are likely too few to be of consequence for cell death, but they are critically important in cancer initiation. Given that there are only three types of damage to the DNA that could be responsible for cell death, this leaves LMDS as the primary cause for cell death (Faw and Shultis 1993).

Strand breakage is also responsible for chromosomal anomalies, some of which were listed in Tables 3-4 and 3-5. DNA strand damage is a serious cellular event; however, the cell comes equipped with chromosomal repair mechanisms. Without them, the damage that occurs to the entire organisms's DNA every day could prove lethal. Chromosomal repair mechanisms provide a mechanism for minimizing the

adverse DNA effects of radiation on the genome, providing that the dose is not so large as to overwhelm the inherent repair mechanisms. However, like many other biological functions, they are not always 100% efficient at performing this task. Single-strand breaks stand a better chance for repair by the cellular DNA repair enzymes (DNA ligase) than do double-strand breaks. First, with single-strand breaks, only one strand of the double-stranded DNA is broken, whereas both strands are broken with double-strand DNA damage. Because one strand is still intact, single-strand breaks are usually stable and within a reasonable distance from each other for repair enzymes to function; however, this is not always the case with double-strand DNA breaks. Secondly, there is a template on the adjacent strand in single-strand DNA breaks to determine where various bases go on the missing strand. The ionizing event that produces a double-strand break may leave the displaced sections close enough together to rejoin with minimal repair, or it may displace large sections, leaving no template for repair enzymes to follow in order to replace the missing segments. Because single-strand breaks in DNA are more easily repaired, cells can tolerate much more of this type of strand breakage before the repair mechanisms are overwhelmed.

Chromosomal aberrations and chromatid aberrations are the two most common types of chromosomal anomalies that can be visibly observed during the metaphase or anaphase stages of the cycle. Chromosomal aberrations are a result of a cell that was irradiated early in the interphase cell cycle (G1 or early S phase), prior to the chromosome being duplicated. Chromatid aberrations are commonly observed when the damage was received in the later stages of interphase (late S or G2 phase) after the chromosome has duplicated and consists of two strands of chromatin. Specific radiation-induced aberrations in chromosome and chromatid structure have been discussed in more depth by Hall (1988). These aberrations may or may not result in the disruption of normal cellular functions, depending on which chromosome the breakage occurred in and where on the chromosome the damage occurred. When examined more closely, the broken ends of the chromosomes appear "sticky" and may fail to mitotically separate with the proper chromatid (Hall 1988). However, when the cell enters a mitotic cycle, these damaged chromosomal units will ultimately fail to replicate properly unless chromosomal repair mechanisms can repair the damage prior to entering mitosis/meiosis. If the repair mechanisms fail, cell death or genetically deficient progeny cells result. Of the single and multiple gene mutations that result, point mutations and small deletions usually involve a small number of bases (~20 to 60), whereas large base deletions or base rearrangements may involve several hundred or many thousands of bases. The mutation frequency increases with the radiation dose (Borek 1993). The cells that undergo more frequent mitotic cycles (intestinal crypt cells, fetal cells, and other rapidly dividing cells) have less repair time, and

a resulting increase in sensitivity to radiation induces genetic mutations and abnormal cell functionality. Cells with less frequent mitotic activity (nerve, lens, musculoskeletal) are conversely less radiosensitive.

The types of genetic damage described above for radiation exposure are also caused by other environmental agents, and their rates are in addition to a high rate of spontaneous production. An average of 200,000 repairs per hour are made to spontaneously occurring damage to DNA in humans. Actually, the damage occurs at a much greater rate than can be observed because the damage is simultaneously being repaired by many physiological mechanisms. Damage is expressed when the rate at which the damage is produced exceeds the body's natural repair mechanisms or when those mechanisms fail.

## 5.4 EFFECTS ON OTHER CELLULAR MACROMOLECULES

DNA is the most critical molecule for damage from radiation. A number of other critical cellular components have been reported; some effects on these molecules are outlined in Table 5-2.

Table 5-2 shows that a wide range of molecules, varying in both size and molecular weight, can be adversely affected by exposure to radiation. The mechanisms by which each is affected are the direct and indirect effects of radiation discussed for DNA. The end results are broken chemical bonds, cross-linkages, and conformational changes. These changes may affect the molecule's biological function; for example, a conformation change in the structure of an enzyme or protein could affect its ability to perform a critical function in a metabolic pathway and thereby halt a certain function.

Amino acids and their larger counterparts, peptides, polypeptides, and proteins, are also susceptible to radiation damage. Irradiation of these molecules frequently results in breakage of hydrogen bonds, disulfide bridges, and cross-linking with DNA or with other proteins. All of these effects can result in conformation changes and alterations in function. Radiation causes the depolymerization of glycogen and cleavage of α-glycosidic bonds within glycogen and other molecules containing α-glycosidic bonds. Glycogenesis and gluconeogenesis pathways within the cell are activated; insulin and blood glucose levels also rise due to increased release of insulin and adrenocorticoid release. By comparison, radiation doses that are orders of magnitude larger than required to produce these effects are used in industry to polymerize monomers to produce hard plastics and bond materials. Even larger doses are required to inactivate bacteria and viruses during sterilization of medical equipment, spices, vegetables, and meat.

**Table 5-2. Some Effects of Ionizing Radiation on Molecules in Animal Tissues**

| Molecule | General effects |
|---|---|
| Amino acids | Production of ammonia, $H_2S$, pyruvic acid, $CO_2$, hydrogen molecules |
| Carbohydrates | Cleavage of glycosidic bonds, depolymerization of monomers, oxidation of terminal alcohols to aldehydes |
| Deoxyribonucleic acid (DNA) | Degradation from base loss and modification, breakage of hydrogen bonds and sugar-phosphate bonds; DNA-DNA and/or DNA-protein cross-linking; single- or double-strand breakage; formation of guanyl, thymidyl and sugar radicals |
| Lipids | Peroxidation and carbon bond rearrangement: conjugated diene formation, aldehyde formation, β-scission, lipid cross-linking, increased microviscosity, cell membrane rupture |
| Proteins | Degradation and modification of amino acids, chain scission, cross-linkage; denaturation, molecular weight modifications, changes in solubility |
| Thiols | Redox reactions, radical formation, cross-linkages, inhibit thiol from mediating damage to lipids |

Source: adapted from Cockerham et al. 1994.

Lipids are ubiquitous macromolecules that participate in a number of cell process. They comprise cell membranes, disruption of which leads to disruptions of homeostasis, cellular dysfunction, and death. Lipids are also involved in the production of prostaglandins, which modulate a number of biological functions, including digestion, reproduction, and neural function. Lipid peroxidation occurs primarily through free-radical attacks at double-bond sites and at carbonyl groups, and starts a chain reaction within cells. When a lipid radical interacts with another organic molecule, that molecule is transformed to a free-radical state which then interacts with another molecule. Given this chain of events, the damage induced after lipid peroxidation can be formidable. Fortunately, animals have several mechanisms by which to slow or stop this chain reaction. A number of free-radical scavengers such as vitamin A, vitamin E, and thiols are available to inhibit the chain reactions. Other detoxification systems that can inhibit the effects of lipid peroxidation include metallothionine, glutathione transferase, reduced NADPH-dependent glutathione reductase, selenium-dependant glutathione peroxidase, ferric manganese and copper-zinc superoxidase dismutases, and catalase. A more in-depth discussion on the specific mechanisms by which each system functions is available (Cockerham et al. 1994). Several of these systems and a number of chemicals have been more closely studied in order to potentially decrease the harmful effects of moderate to high doses of radiation in humans and animals, but results have been mixed (Biambarresi and Walter 1989).

## 5.5 MECHANISMS OF CARCINOGENESIS

The exact mechanism(s) by which cancer is produced are not clearly understood, but over the years, several theories and models have been developed that describe the events that scientists suggest must take place in order for cancer to occur. Some of the more traditional carcinogenesis models are briefly summarized in Table 5-3. A number of factors have been identified (such as diet; hormonal status; genetics; and exposure to some solvents, chemicals, and ionizing radiation) that appear to predispose some individuals to developing cancer. Both chemicals and ionizing radiation are known to induce many types of cancer and much of the evidence for this observation was discussed in Chapter 3 of this profile.

Cancer is the major latent effect after exposure to radiation, with the critical molecule being the DNA. Cells depend on their DNA for coding information to make very specific enzymes, proteins, hormones, vasoactive substances, and a host of other chemicals in order to live. When the genetic information containing the "blueprint" for these substances is disrupted, cell homeostasis is disrupted, with a wide range of carcinogenic and non-carcinogenic toxicological effects that have been described in Chapter 3.

Not all alterations in the genome will result in the expression of immediate adverse events. Radiation may cause genetic damage, which includes gene deletions, point mutations, frameshift mutations, and "nonsense" coding of some genes on one or many chromosomes. These alterations occur by the same direct and/or indirect mechanisms outlined in Section 5.3. If these genes are not used by the cell or if their mutation or total absence is of little consequence to normal cell function, no immediate effects may be incurred. Cell function and homeostasis is not disrupted. These seemingly inconsequential genetic effects may initially be of minimal importance. However, with spontaneous changes in the genetic apparatus of somatic cells continuing over time and with further exposure to environmental carcinogens, the amount of misinformation in the genetic apparatus continues to increase within the cell's DNA. If this misinformation affects the DNA coding that either controls or suppresses an oncogene, then oncogenic lesions may be initiated.

The formation of cancer has been an area of intense research in the scientific community for centuries. In 1775, Percival Pott was the first to report that cancer could be caused by environmental factors. Pott described a number of cases of cancer in men employed as chimney sweeps sometime during their life. Pott concluded from his observations that their exposure to soot was in some way related to their developing

**Table 5-3. Some Models That Describe the Induction of Cancer in Animals**

| Model | Model type and premise | Model characteristics |
|---|---|---|
| Single Hit [a] | Mechanistic model: One "hit" is sufficient for a cell to mutate and then transform into a neoplastic cell. | Tumor development depends only on the total dose received and not on the pattern of exposure; yields high estimations of risk compared to other models |
| Multi-Hit | Mechanistic model: A critical number of hits must occur before the cell becomes neoplastic. | May produce very high or very low "safe-dose" estimates; doesn't easily account for dose-response relationships that are linear at low doses; begins to curve as dose increases |
| Multistage/ Linearized Multistage (LMS) | Mechanistic model (based on the model of Armitage and Doll 1957): A progression of orderly events must occur in a cell in order for cancer to occur. | Use of upper bounds results in a model less sensitive to changes in data; multi-degree polynomials are fitted using only 2 or 3 dose levels; a constant dose rate is assumed (which is not always the case); does provide conservative risk estimates |
| MVK | Mechanistic model; Two-stage model: Similar to the LMS, but it assumes that altered cells have a selective advantage over normal cells. | Assumes tumors come from mutations of anti-oncogenes; assumes 2 events must occur for malignant transformation;<br>allows for cell kinetic information and mutations to be incorporated into the model |
| Probit | Statistical model | Estimates probability of a response at a given dose; may not reflect scientific observations of dose-response when extrapolating from a 50% response dose to a 1/1,000,000 risk estimate |
| Logit | Statistical model | Derived from chemical kinetic data; used to derive "virtually safe doses" by some government agencies until the late 1970's; similar to Probit model |
| Weibull | Statistical model | Used to derive "virtually safe doses" by some government agencies until the late 1970s; uses power transformations to describe the data; greater flexibility than either the Probit or Logit models; risk estimates range between the LMS and multihit mechanistic models; dose and time relationship are described |

[a] A "hit" is defined as a critical cellular interaction, such as a gene mutation, that alters the cell's DNA (Faustman and Omenn 1996).

Source: summarized from Faustman and Omenn 1996 and Rees and Hattis 1994

cancer of the scrotum. Since that time, a number of chemical, environmental, and lifestyle factors have been identified as either be directly or indirectly implicated in producing different types of cancer. Many of these chemicals have similar physico-chemical and structural characteristics.

Today, the induction of cancer from exposure to some chemicals is believed to be a multi-stage process that involves at least three distinct phases and multiple steps. Some chemicals or agents may be capable of inciting one, two, or all three of these steps. It is believed that exposure to ionizing radiation involves the same multi-step process as does the chemical carcinogen exposure, and that radiation can induce each

step. The first stage is initiation, which is characterized by the fixation of a somatic mutational event in the cell's DNA. This damage may occur by direct, indirect, or a combination of the events described in Sections 5.2 and 5.3. This initiation may occur at one or multiple sites within the genome and may affect any gene on any chromosome in any exposed cell. Once exposed, certain outcomes are possible. In cases of high doses of radiation, the cell may sustain such extensive genetic damage that the cell is unable to perform its functions or sustain itself metabolically, in which case it simply dies. High doses may kill so many cells that the organism shows signs and symptoms, but lower doses can kill few enough cells that effects are not readily observed. The cell may attempt to repair the damage using alkyltransferases, base excision repair, nucleotide excision repair, mismatch repair, or other innate repair mechanisms inherent to that cell. If all of the damage is repaired correctly, the cell is considered normal and not at risk for developing cancer. However, repair mechanisms are not always 100% effective and may result in incorrect repair or no repair at all. In this case, the cell may either live and tolerate the damage to the genetic material or undergo apoptosis (programmed cell death). Only the cells that continue to live and reproduce can potentially produce cancer. Exposure to ionizing radiation can result in changes to a cell's genetic apparatus and can act as an initiating agent in the development of cancer. Additional information about the ability of radiation to inflict damage on the DNA structure is presented in Chapters 2, 3, and earlier in this chapter.

Initiation requires at least a partial failure of gene repair mechanisms and one or more cell mitotic cycles before the genetic alteration can be "fixed" into place. Initiation is an irreversible process once this fixation occurs. Whatever the mechanism, the end product is a mutation of the cell's DNA that the cell's innate repair systems failed to restore to the normal genetic state. This mutation is considered an adverse event; however, the initiation or "genetic recoding" alone is not sufficient to produce cancer.

The initiation step must be followed by the second stage, promotion. A promoting agent is one which stimulates the initiated (or pre-neoplastic) cell to divide or otherwise provides certain conditions that allow the preferential selection of mutated cells to survive over unmutated cells in the tissue. In contrast to initiation, the promotion step is a reversible step both at the DNA and cellular level, and depends on continuous exposure to the promoting agent. This reversibility is a characteristic of the promotion stage of carcinogenesis. For promoting agents, there is no evidence to suggest that these chemicals or other factors must interact directly with the DNA to affect cell proliferation. Promoters need not necessarily be carcinogenic agents themselves. Many chemicals (phenobarbital, dioxins, cholic acid), as well as some hormones (estrogen and thyroid-stimulating hormone) are not carcinogenic themselves, but have been

found to promote carcinogenesis after certain cells have undergone the initiation process in some species of animals. Promoting agents may also be species specific. A promoting agent for cancer in laboratory animals may not necessarily be a promoting agent in humans. Although some promoters are actually non-carcinogenic when administered by themselves, other promoters can act as both initiators and promoting agents. Radiation is an excellent example of an agent that can act as both an initiator (producing gene mutations and chromosomal alterations) and as a promoting agent by stimulating cell division after exposure.

The last stage of carcinogenesis is called progression. Progression agents cause uncontrolled and extensive proliferation of abnormal cell types. During this stage, a specific phenotype of mutated cells is selected that effectively evades the host defense mechanisms and then undergoes massive proliferation. Arsenic salts, asbestos fibers, benzene, benzoyl peroxide, and hydroxyurea have all been identified as proliferation agents associated with cancer formation. This uncontrolled and extensive proliferation of abnormal cell types leads to the formation of solid tumors (adenomas, squamous cell carcinoma, adenocarcinomas, etc.) or non-solid tumors (leukemia, lymphoma, etc.) at one or multiple locations throughout the body. How locally invasive the tumor is (aggressiveness) or the ability of the tumor to relocate to sites distant from the site of initial formation (metastasis) depends on the type of tumor formed. If the progression becomes widespread throughout the body or causes severe harm to vital organ functions, the organism will eventually succumb to organ failure and die. Radiation is capable of acting as a proliferation agent in the formation of cancer in the skin of mice.

Gene mutation is a key step in the formation of cancer. Any gene or any locus on the DNA can be affected by a genotoxic agent and undergo. Certain gene mutations and chromosomal irregularities are associated with specific cancers in humans and laboratory animals. These genes are called proto-oncogenes, oncogenes, and tumor suppressor genes. Proto-oncogenes are similar to viral oncogenes. Proto-oncogenes are considered normal genes. When mutated, proto-oncogenes become oncogenes, which, in turn, initiate carcinogenesis. Both proto-oncogenes and oncogenes are dominant genes that normally function to regulate cell growth, signal transduction, and nuclear transcription (Pitot III and Dragan 1996). Mutations in these genes result in the activation and subsequent neoplastic transformation of cells containing these mutated genes. Conversely, tumor suppressor genes are recessive genes which normally function to slow cell growth. When these genes mutate, cells lose this capacity to down-regulate cell growth, which results in the activation and subsequent neoplastic transformation of the mutated cells. Radiation may cause mutations in proto-oncogenes, oncogenes, and tumor suppressor genes.

Environmental factors have also been shown to play a role in lung carcinogenesis, particularly in the promotion stage. These environmental factors include tobacco, silicon dust, diesel fumes, and possibly other toxicants found in the breathable air of mines. Other factors that have been related to chemically-induced cancers include alcohol use, food additives, diet, sexual behavior, occupation, air and water pollution, pharmaceuticals, and bacterial and viral infections.

## 5.6 IDENTIFICATION OF DATA NEEDS

The following has been identified as a potential data need regarding health effects associated with exposure to ionizing radiation.

Environmental factors, such as tobacco, silicon dust, diesel fumes, and possibly other toxicants found in the breathable air of mines, together with radiation, have been shown to play a role in the development of lung cancer. More research is needed on biomarkers as identifiers of unique DNA and cellular changes associated with exposure to radiation. More research is also needed to determine possible interactions between other carcinogens and ionizing radiation.

Human epidemiological studies have clear limitations in the low-dose range, and mechanistic studies may be important in further clarifying the true effects at low doses. In this regard, there is a need to more fully understand the role of DNA repair at low dose, gene expression in carcinogenesis, and the role of radiation in cancer promotion and progression. Mechanistic studies should be considered for non-carcinogenic effects such as human developmental radiobiology, particularly for internal emitters.

## 5.7 SUMMARY

This chapter summarized the major mechanisms by which ionizing radiation exerts it toxic effects on cell structure. Macromolecules, in particular DNA, are the critical molecules for damage from radiation. The method by which radiation interacts with a biological medium may be direct or indirect. Damage can occur due to direct ionization of the DNA molecule itself or indirectly through the formation of toxic products, such as free radicals, hydrogen peroxide, hydroperoxy radicals, and hydroperoxy ions, that diffuse from the site of formation and interact with any molecules in their path. Since cells rely heavily on their DNA for instruction information, when the genetic information containing the “blueprint” for this information is disrupted, cell homeostasis is disrupted, and a wide range of biological responses is

encountered. These responses include non-carcinogenic and carcinogenic end points. Other molecules, such as lipids, proteins, thiols, amino acids, and carbohydrates, can also be damaged when irradiated. A number of models were presented that reflect possible mechanisms of cancer induction, as well as a brief discussion of the three steps of cancer formation. By knowing the specific mechanisms by which radiation produces carcinogenic and non-carcinogenic end points, research can focus on identifying biomarkers of effect with which to better assess the effects of low-level radiation exposure.