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Cellular and Molecular Research to Reduce Uncertainties in Estimates of Health Effects from Low-Level Radiation

A Feasibility Study

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ABSTRACT

A study was undertaken by five radiation scientists--a *cytogeneticist*, a *cell biologist*, a *health physicist*, a *mammalian biologist*, and a *molecular biologist*--to examine the feasibility of reducing the uncertainties in the estimation of risk due to protracted low doses of ionizing radiation. The idea that such a study would be timely was derived from the discovery of oncogenes, the development in the past 15 years of the techniques of recombinant DNA molecular biology, and the evident and significant progress that has been made in the characterization of certain human cancers in genetic terms.

In addressing the question of feasibility, a review was made by the study group: of the cellular, molecular, and mammalian radiation data that are available; of the way in which altered oncogene properties could be involved in the loss of growth control that culminates in tumorigenesis; and of the progress that had been made in the genetic characterizations of several human and animal neoplasms. On the basis of this analysis, the study group concluded that, at the present time, it is feasible to mount a program of radiation research directed at the mechanism(s) of radiation-induced cancer with special reference to risk of neoplasia due to protracted, low doses of sparsely ionizing radiation.

To implement a program of research, a review was made by the study group of the methods, techniques, and instruments that would be needed. This review was followed by a survey of the laboratories and institutions where scientific personnel and facilities sufficient to participate in a significant part of the program are known to be available. It was noted that this survey was not exhaustive nor was it intended to preclude the participation in the program of additional research groups that may be capable of effectively doing so. A research agenda of the principal and broad objectives of the program is also discussed.

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- o Robert A. Alexander, former Chief of the Radiation Protection and Health Effects Branch, who recognized the limitation of epidemiologic and animal studies in estimating health risks from low-level radiation, and the potential for reducing those uncertainties through cellular and molecular studies of the mechanisms involved in the development of human cancer;
- o Alan K. Roecklein, Dr. Donald A. Cool, and Dr. Bill M. Morris, whose leadership and support made this feasibility study possible; and
- o Dr. Shlomo S. Yaniv, the NRC Project Manager, whose diligence, advice, and support kept the study team moving ahead in previously unexplored waters.

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EXECUTIVE SUMMARY

Although it is generally acknowledged that cancer is the major hazard to health from low doses of ionizing radiation, appreciable uncertainty exists on the degree of hazard particularly for protracted exposures. This uncertainty reflects the complexity of the variety of patho-biological states that constitute different cancers, but, more importantly, a lack of an adequate understanding of the molecular mechanism(s) of cancer induction particularly by radiation.

However, the discovery of oncogenes, and the development of the techniques of recombinant DNA that contributed to it, are leading to an understanding of human cancer in fundamental, molecular terms. These advances hold out promise that, in the near term, major advances can be made in the molecular characterization of the abnormal *growth states* of specific neoplasms. It is not known how radiation induces transitions to and between such states. Still, for the first time, the possibility exists that the essential steps in radiation-induced cancer can be unravelled.

It does not necessarily follow that the success of a program of radiation research on oncogenic mechanisms will automatically result in a narrowing of the uncertainties of cancer induction by protracted, low doses of radiation. However, as a result of this study, it is the belief of the study group that reductions are not possible without such an understanding but are likely when oncogenic/molecular mechanisms become more fully understood.

The work of the study group was divided into six parts each containing its own summary, and a set of illustrations, tables, and references, as appropriate. A glossary is included at the end of the report. Synopses of each part follow.

Task 1: Radiation-Induced Mutagenesis and Oncogenesis *In Vivo*

In either humans or animals, it is not possible to distinguish between a neoplasm that is caused by ionizing radiation and one that occurs "spontaneously," that is, from some unknown cause. In essence, radiation-induced neoplasms can be recognized in any population by statistical means only, by the identification of an excess frequency above the natural incidence (BEIR III Report, 1980; UNSCEAR Report, 1988; BEIR V Report, 1990). Neoplasms can be induced in virtually any tissue or organ, however. Some tissues are more sensitive to radiation-induced neoplasia, in both animals and humans and include bone marrow, breast, lung, and thyroid gland tissues (see Table 1.1). To improve an understanding of the risks due to low doses at low dose rates, it is logical that research should concentrate on these tissues.

Neoplasia develops as a multistage process that proceeds, operationally at least, from normal to neoplastic cells (see Figure 1.1). The first step, *initiation*, reflects a genomic change which produces a transformed cell, that is, one that has altered phenotypic characteristics and frequently can be differentially stimulated to form a focal proliferation of cells. The second step, *promotion*, enhances the formation of a neoplasm in an initiated cell(s) by encouraging proliferation and clonal expansion, that is, the selective accelerated growth of transformed cells. Further steps, included in a process called *progression*, confer new properties on promoted cells and lead to

sequential phenotypic changes. The latter are thought to involve additional genetic changes and progressive increases in degrees of malignancy.

A number of biological characteristics can affect radiation oncogenesis. In both people and animals, age at exposure is a major determining factor. The general rule is that exposures at earlier ages increase lifetime risks of many types of neoplasia including leukemias, thyroid neoplasia, and mammary neoplasia (UNSCEAR Report, 1988; BEIR V Report, 1990). Sex of the exposed individual also is a major factor for some types of radiation-induced neoplasms reflecting, at least to some degree, hormonal influences. Associations between increased levels of the mammary gland hormone prolactin and increased risk of breast cancer, and between increased levels of thyroid-stimulating hormone and increased risk of thyroid cancer, are well documented. In some species, hormonal makeup can influence leukemia induction by radiation, as well.

With respect to low-dose radiation tumorigenesis, the same problems of interpretation and significance exist for animal data as for human data. That is, most studies have been performed with either high doses of a low-LET radiation, or with lower doses of a high-LET radiation because of its greater biological effectiveness. There are relatively few data on low-dose, low-LET tumor induction. Even for only low-LET exposures, one particular shape of dose-response curve does not fit all of the data for the induction of different types of tumors. The effectiveness per unit dose generally increases with LET. Decreasing the dose rate decreases the effectiveness for low-, but usually not for high-LET radiations.

Task 1: Appendix A

There have been few large epidemiological studies of the risks of radiation neoplasia in human populations which have followed the exposed populations until the end of life. As a result, there are substantial uncertainties in both the shape of the dose-response curves and lifetime risks. The uncertainties relative to low-dose, low-dose-rate exposures are large because the only populations for which statistically meaningful results have been demonstrated are those which were exposed to relatively large doses and usually at high dose rates. The size of the population required to provide statistically meaningful results is approximately inversely proportional to the square of the excess cancer. Thus, if 1,000 samples (human or animal) in both the irradiated and control populations were required to provide adequate statistical power at 1 Gy (100 rad), about a billion in each group would be required at 1 mGy (100 mrad; BEIR III Report, 1980, p. 140). Such large population requirements generally prohibit attempts to resolve the risks of radiation at low doses and low dose rates due to the inordinately large costs, and lengths of the studies, that would be involved.

A major factor in the recent reassessments of the risk of radiogenic human cancer was the reevaluation of the radiation dosimetry for the Hiroshima and Nagasaki A-bomb survivors, a study that essentially was completed in 1987. These changes are important because most of the current estimates of risk of radiogenic cancer have been derived from the Japanese data.

Task 2: **Mutation, Neoplastic Transformation, and Tumor Formation** **Molecular Mechanisms**

In the past 10-15 years, significant technical innovations have been introduced into the study of the cellular and molecular properties of mammalian cells including human cells. To methods for the growth of mammalian cells in culture, which were first applied some 15 years earlier, genetic engineering/DNA recombination technology were added. As a result, it has become feasible to address fundamental questions in biology at a molecular level not achievable in the past. One such question has been the molecular/genetic basis of uncontrolled cellular growth, cancer.

In operational terms, neoplasms are thought to be the result of the three principal steps as noted--*initiation*, *promotion*, and *progression*. These steps are described in terms of the basic molecular and cellular processes that are believed to be responsible for them. Because a tumor results from one or more heritable changes in the progenitor cells of the tissue of origin, the mutation of genes called *oncogenes* is identified as a driving force in the development of cancer. Radiation, like other genotoxic agents, is known to damage DNA and is recognized as a mutagen/carcinogen. However, cells have the ability to modify genetic damage and, consequently, repair/misrepair processes are important in the expression of the changes that are induced.

Because uncontrolled cellular growth is the feature that unifies neoplasms, a number of different genetic changes have been identified for their ability to play a role in the process. These changes occur in a set of normal genes, called *proto-oncogenes* some of which have dominant and others recessive action. The identification of these genes with neoplastic growth was first made because of their involvement with the tumorigenic and molecular properties of certain RNA-containing viruses called *retroviruses*. The constitutive forms of the some 50 proto-oncogenes that have been identified have normal roles to play in cells. But when they are altered, as in the instance of a mutated form incorporated into the genome of a cell via a retrovirus, they can lead to uncontrolled cellular growth, or to a lack of control of differentiation, such that a neoplasm is the result. Radiation, and other physical and chemical agents, are also able to mutate oncogenes and thus to give rise to the chain of events that culminate in cancer.

Through the application of DNA recombinant technology, a number of alterations in proto-oncogenes, or in the control of their expression, have been associated with the neoplastic transformation of cells. Hence, a variety of molecular mechanisms can culminate in an aberrant regulation of growth. Although examples exist of the association of neoplastic transformation with a mutation in a single proto-oncogene, there is evidence that two or more altered oncogenes may collaborate in the processes involved in tumorigenesis. For example, it has been proposed that as many as 10 alterations may be involved in small-cell carcinoma of the lung.

Proto oncogenes, like other genes, are transcribed into RNA from which a messenger form is produced that is then translated into proteins including the enzymes that catalyze various reactions. The products of proto oncogenes, the proteins, have a variety of functions; they have been demonstrated to influence growth by controlling processes in other cells, or by acting in the cell of origin at its outer membrane, in its cytoplasm, or in its nucleus. The products

of oncogenes are also known to influence the communication between adjacent cells changing thereby the societal properties of a cell.

Oncogene changes are also thought to be involved in the steps of *promotion* and *progression*. Promotion involves the clonal expansion of a transformed cell, and progression is associated with the ability of transformed cells to invade other tissues and to metastasize. Because radiation-induced neoplasms are indistinguishable from those that arise spontaneously, or are induced by some other etiological agent, and because of the mutagenic properties of radiation, it is likely that radiation can affect all or the steps in neoplasia. It is also possible, therefore, that the effects of radiation may interact with those of other environmental agents.

Task 3: Elements of a Radiation Research Program, Cellular/Molecular Mechanisms in Cancer Induction

This Task has four essential parts. The first is a critical review of the information that is available, primarily from cell and animal studies, that is connected with the mechanism of radiation-induced oncogenesis. The second is a review of what has been discovered of the roles of genes, particularly oncogenes, in radiation-induced endpoints. The third is an analysis of what is known about the role of oncogenes in human neoplasia. And the fourth is a prospectus of the scope of a radiation research program to reduce the uncertainties of the risks of cancer from low doses of radiation. In addition, gaps in our knowledge are identified in reference to the topics that are important to the understanding of radiation-induced cancer.

Cellular Neoplastic Transformation and Tumorigenesis in Experimental Systems

Although the kinds of cells that have been available for experimental work have been limited, significant insights have emerged connecting the radiobiology of neoplastic transformation *in vitro* and tumorigenesis *in vivo* as follows:

- 1) The dose dependence of tumor induction reflects a competition between the transformation events and the lethal events in the target cells of a given tissue (Fig. 3.1 and 3.2). To be the progenitor of a population of cells that constitute a neoplasm, an irradiated cell must survive. In a number of instances, the net effect of these two processes is a bell-shaped curve of the dependence of tumor induction on dose (Fig. 3.2).
- 2) As for other endpoints, the efficiency of the neoplastic transformation of cells, and the induction of tumors, increases with LET. In general, a high-LET radiation produces larger frequencies per unit dose than does a low-LET radiation. However, because cells are generally killed more efficiently with a high- vs. a low-LET radiation, cell killing can have a more pronounced effect with the former. Thus, the bell-shaped curve of tumor induction with a high-LET radiation is generally shifted toward lower doses compared to that with a low-LET radiation (Fig. 3.2).
- 3) In addition to the influence of repair relative to cell killing, repair/misrepair can significantly alter the radiation-induction process. In the low dose region, where cell killing is minimal, reducing the rate of exposure to a low-LET radiation generally results in a reduction in the initial slope of the cell transformation or the tumor induction curve.

With low dose rates of a high-LET radiation, such as fission-spectrum neutrons, the situation is less general. In some instances, protracting the dose has increased yields of transformed cells or tumors, and in others it has not or it has reduced them. A generalization in respect to the influence of dose protraction of high-LET radiations is not possible at the present time.

- 4) Although the initial yield of neoplastically transformed cells is significantly reduced at reduced rates of a low-LET exposure, the net yield of transformants can be appreciably increased by the action of a compound known as a *promoter*, e.g., a phorbol ester, applied after the irradiation. Hence, the possibility exists that other agents in the environment may interact with radiation effects to alter the yield of transformed cells and therefore tumors due to a given radiation experience.

The analysis of *in vitro/in vivo* data made evident a number of gaps in our knowledge and the principal deficiencies were discussed.

Genes and Oncogenes in Radiation Endpoints

The power of the genetic methods that has emerged from the application of DNA recombinant techniques in other areas of research led quite naturally to the application of these methods in radiobiology. Some progress has been made, but the amount of information available thus far is limited. The current situation is evident from the following.

- 1) The survival of cultured cells can be influenced by the *ras* oncogene, but the basis for this effect has not been worked out. Similarly, it appears that the application of a growth factor, like epidermal growth factor, can alter the survival response of certain cells.
- 2) Radiation is an effective mutagen. However, in contrast to chemical mutagens, the heritable changes that are produced by radiation generally are due more to chromosomal breakage-rearrangement events, *extragenic mutations*, than to point effects, *intragenic mutations*.
- 3) Although specific genes appear to be induced by radiation in transformed cultured cells, they do not appear to be among the known oncogenes.
- 4) Progress has been made on the roles of some oncogenes in tumors induced in experimental animals particularly in respect to acute myeloid leukemia in the mouse. The need for the activation, in some instances, of at least two oncogenes has also been noted.

In reference to the endpoints discussed above, a number of gaps were evident and these were discussed as subjects for additional research.

Growth State Characterizations of Human Neoplasms

The fruitful application of the methods of contemporary molecular biology are no more evident than in the study of oncogenes in human cancer. Significant advances have been made in characterizing, in specific types of tumors, the mechanism of action of those oncogenes that appear to be activated as well as in

locating them in the chromosomes of human somatic cells (see Tables 2.1, 2.3, and Fig. 2.3). The following points were brought out:

- 1) Chromosomal rearrangements and/or deletions, as well as the activation of particular oncogenes, are involved in specific spontaneous neoplasms. The oncogene/chromosomal data support the hypothesis that human tumors are clonal in origin.
- 2) A correlation appears to exist between the number of genetic/chromosomal alterations in a given type of neoplasm and the latency period for its appearance; tumors that have few changes appear earlier after induction than those that have many.
- 3) In some instances, genetic/chromosomal characterizations have been sufficiently worked out to facilitate an association between specific histological states with specific characterizations, e.g., Burkitt's lymphoma, chronic myeloid leukemia, and colorectal carcinoma.
- 4) Some human tumors are believed to require a number of essential changes. As a consequence, it is not clear what is cause and what is effect and, therefore, which changes are independent and which dependent.

Although the progress in characterizing spontaneous human neoplasms is significant and its rate is accelerating, what has been accomplished thus far in most instances is to describe rather than to explain. Thus, the oncogenic/chromosomal descriptions are a kind of taxonomy. In addition to the need for similar characterizations of all radiogenic human tumors, a number of fundamental questions are evident. Principal among these is if, and how, the transitions between successive growth states are produced by radiation. This gap and others are identified.

A Radiation Research Program

Numerous factors, in addition to those that are radiobiological, are known, or suspected, to play a role in influencing radiation oncogenesis. In sum, these can be expected to underlie the uncertainties in the epidemiology of cancer including radiation-induced cancer. The recently issued BEIR V Report (1990), in which estimates of some radiation risks were significantly increased, illustrates the need to modify earlier estimates partly because of the lack of a coherent set of ideas of how cancer is induced by various etiological agents including radiation. In view of the apparent complexity of the problem, one must inquire: Is it feasible to reduce the uncertainties in risk estimates of radiation-induced neoplasms based upon an expanded knowledge of the underlying mechanism(s) of the induction of unregulated cellular proliferation *in vivo*?

The answer to this question is a qualified "yes." This optimism is based largely on:

- 1) The dramatic developments that are occurring in the understanding of the oncogenic basis of human cancer;
- 2) The assumption that growth-state characterizations will be completed for all of the radiogenic human cancers of interest; and

- 3) The assumption that the molecular biology of the radiation-induced transitions between growth states will become understood.

In addition, to accomplish a significantly improved understanding of radiation oncogenesis, so that the uncertainties in radiation risks may be reduced, three further objectives are identified:

First, a mechanistic framework is needed to relate the oncogenic bases of radiation-induced cancers with those of spontaneous cancers;

Second, growth-state characterizations, and the radiobiology of transitions between them, will have to be worked out for animal tumors just as is being done for human neoplasms; and

Third, strategies must be validated that will enable extrapolation of experimental data on tumorigenesis to people.

Although differences are anticipated between the oncogenic changes in cells that culminate in human as opposed to animal cancer, it is quite likely that essential similarities exist. For example, the oncogenic basis for the uncontrolled growth of animal and human myeloid cells, which becomes manifest as leukemia, is expected to result from mechanisms that are similar in essence. Hence, an understanding of mouse leukemia should facilitate the understanding of human leukemia.

Task 4: Methods, Techniques, and Instruments for Mechanistic Studies

Although many technical innovations for the study of cellular and molecular biology have been introduced in the past 10 to 15 years, effectively to mount a research program in radiation oncogenesis further developments are needed. These include improvements in:

- 1) The measurement of the various types of lesions in genomic DNA particularly in reference to their production, repair, and misrepair;
- 2) The techniques for the cultivation of human and animal cells *in vitro* so that the properties of the types of cells of interest can be quantitatively studied in reference to mutation and neoplastic transformation;
- 3) The improvement and application of rapid methods of image analysis for the study of chromosome aberrations; and
- 4) The expanded use of cell-animal systems, including the use of transgenic animals, so that the experimental facility of cells in culture can be effectively coupled to the *in vivo* influences that are relevant to tumorigenesis.

Efforts to improve the materials and methods that are currently available also should include the need to measure changes induced by low doses, and after protracted exposures as well. Although studies of the mechanisms of radiation oncogenesis do not have to be exclusively pursued in the context of "weak signals," critical issues, like the presence or absence of thresholds, may have to be examined in the dose region of primary interest. Hence, the techniques for

the measurement of molecular and cellular changes should be improved to permit the sensitivity and resolution that would be required.

In the last analysis, radiation-induced neoplasms can only be demonstrated *in vivo*. For this reason, tumor formation in experimental animal systems has to be anticipated as an essential endpoint in a radiation research program. It is not recommended, however, that large-scale experiments, simply to measure the dose dependence of tumorigenesis, should be undertaken. Animal measurements, including the use of *xenographs* to measure neoplastic properties induced in human cells, should be undertaken only when they are designed to address relevant cellular/molecular questions.

Task 5: Facilities for a Radiation Research Program

As in any area of science, the success of a program of radiation research will be dependent, in the first instance, upon the availability of qualified investigators. In addition, a range of facilities are required. Not only the laboratories and equipment typical of what has been available from the 1950's are required, but also the facilities and instruments that have emerged during the past 15 years as concomitants of new developments in cell and molecular biology. Principal among the latter have been those that enable the determination of changes produced in the genetic code of a cell.

In this Task, the major types of experimental procedures in a program of research are noted and categorized, the kinds of laboratories in which these procedures could be undertaken are described, and the operating units that are generally recognized to have the facilities, as well as the personnel, effectively to pursue one or more parts of an integrated program are listed. In the latter connection, it is important to recognize that the list is not intended to be exhaustive nor to suggest that scientists at other laboratories could not make effect contributions to the program.

Task 6: Agenda for a Radiation Research Program, Topics, Methods, and Facilities

In Task 6, the broad and principal objectives of an agenda for a radiation research program are identified. These were derived from the progress that has already been made in describing the oncogenic basis of human cancer plus the innovations in DNA technology that gave rise to them. The need for further information to characterize, in terms of oncogenes, the growth states of human neoplasms is discussed, and the further requirement of an understanding of how radiation causes transitions between successive growth states is emphasized. The relevance to radiation issues of other biologically-active environmental agents is pointed out, and the need for a rational basis for extrapolating cell and animal data to people is noted. Lastly, in addition to program objectives, the potential usefulness of improvements in methods and instruments that could enable the rapid screening of biological material is discussed.

A program of radiation research would play an effective role in improving the overall understanding of human cancer, and would have the potential of reducing the uncertainties in risk due to protracted low doses of radiation. Because of the many benefits to industrialized society that are derived from the use of radiation and the devices that give rise to it, the desirability of reducing the ratio of risk to benefit via a program of research was noted.

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Task 1

"Radiation-Induced Mutagenesis and Oncogenesis *In Vivo*"

1.1 INTRODUCTION

In this Task, the extensive and diverse literature on radiation-induced neoplasia is reviewed. Human and animal studies will be summarized with emphasis on data that relate to concepts of radiation risks and the mechanisms that play a role in radiation oncogenesis.

The question of how data from animal studies relates to radiation oncogenesis in people is particularly relevant. The evidence is clear that, for people, some tissues are at greater risk than others, e.g., breast, bone marrow, colon, lung, and thyroid. In animals, these same tissues have also been shown to be sensitive to radiation oncogenesis.

1.2 RADIATION ONCOGENESIS, GENERAL PRINCIPLES

To arrive at an understanding of the cellular and molecular bases of oncogenesis it is necessary to understand the oncogenic process in tissues and organisms. Basic biological relationships in oncogenesis are reviewed in this section.

1.2.1 Nature of Radiation-Induced Neoplasia

On the basis of the causative agent, neoplasms of a given histology in a given tissue are generally indistinguishable. Still, there are significant differences in the types of neoplasms which occur in different tissues in a single species, or in the same tissue in different species.

1.2.1.1 Comparison of Spontaneous and Radiation-Induced Neoplasia

In either human beings or animals, it is not possible to distinguish between a neoplasm that is caused by ionizing radiation and one that occurs "spontaneously," i.e., which occurs from some unknown cause. In essence, radiation-induced neoplasms can be recognized in a population only by statistical means because it is necessary to observe a frequency in excess of the natural incidence (BEIR III Report, 1980; BEIR V Report, 1990).

1.2.1.2 Tissue and Species Specificity

Neoplasms can be induced in virtually any tissue or organ of the body if the exposures are adjusted appropriately; however, it is clear that there are significant differences in the oncogenic effects of radiation in different tissues. In people, tissues of the thyroid gland, breast, bone marrow, colon, and lung are the most sensitive (BEIR III Report, 1980; Storer, 1986; BEIR V Report, 1990). Among the least sensitive tissues are the prostate, testis, uterus, and uterine cervix. Even within the human hematopoietic system, tissue sensitivity varies; chronic lymphocytic leukemia and Hodgkin's disease are refractory to induction by radiation, whereas acute lymphocytic leukemia, the granulocytic leukemias, and multiple myeloma contribute to the high oncogenic sensitivity of the bone marrow.

In animals, the sensitivity of different tissues varies by species and by strain. Although there are certainly some uniquely responsive tissues in some animals, such as the tissues of the Harderian gland (Fry et al., 1982) and the ovary (Watanabe et al., 1986) in mice, in general, the same tissues that are found to be sensitive to ionizing radiation oncogenesis in people are also found to be sensitive in a variety of animal species (see Upton, 1986).

Many different animal models have been used to study experimentally the effects of radiation on neoplastic induction. The three most widely used have been various strains of laboratory mice and rats, and the beagle dog. However, many other studies have been conducted with rabbits, guinea pigs, Syrian and Chinese hamsters, non-human primates, and even larger domestic animals such as the pig and burro (Goldman and Bustad, 1972; IAEA, 1976; IAEA, 1978; Upton et al., 1986; Thompson and Mehaffey, 1986). There is considerable variation in the responses of the various species, and even among different random bred or inbred strains of the same species, such as the mouse.

It is important to recognize that the evaluation of radiation-induced neoplasia in any given species must be made in the light of the natural incidence of particular types of cancer in that species. In both human beings and animals, tumors of the tissues most sensitive to radiation oncogenesis are not necessarily the types that occur with the highest incidence in the population overall. For example, in people, the thyroid gland is highly responsive to radiation, yet thyroid tumors occur spontaneously with relatively low frequency (see Table 1.1, from BEIR III Report, 1980). In dogs, myeloid or granulocytic leukemias rarely appear spontaneously, yet they are readily induced by radiation (Dungworth et al., 1970; Fritz et al., 1986). Conversely, prostatic cancer in men is very common, yet the tissue of this organ appears to be refractory to radiation induction (BEIR III Report, 1980). Despite such examples, it has been found that, in general, susceptibility to radiation-induced neoplasia, at least in some species such as the mouse, is directly proportional to the natural incidence in the population (Storer et al., 1988). This generalization has important implications for risk estimation.

It is not known whether or not the varied susceptibility of different tissues to the induction of cancer by radiation is due to a tissue-dependent difference in the likelihood of occurrence of the initial molecular/cellular events or to differences in expression of these events as a consequence of a variety of other factors (Fry and Storer, 1987).

1.2.1.3 Extrapolation of Animal Data to Humans

If the susceptibility to radiation-induced neoplasia is proportional to the natural incidence of that disease, then the relative risk model may be the most appropriate one to use for risk estimation. Risk estimates of radiation-induced human and rodent tumors were compared and it was found that the relative risks for lung, breast, and leukemic tumors were not significantly different (Storer et al., 1988). Because these are three of the tissues of primary concern for low-dose oncogenesis in people, this relationship is a useful finding.

There has always been controversy about the validity of extrapolating from animals to people. There are, however, many reasons why it is critical to evaluate human risks in the light of data from animal studies. Many different factors influence radiation responses (see Table 1.2, from Fry and Storer, 1987). The amount of information available on these factors from human data is varied

Table 1.1 Sensitivity of Various Tissues to the Oncogenic Influence of Radiation

<u>Site or Type of Cancer</u>	<u>Spontaneous Incidence</u>	<u>Relative Sensitivity to Radiation Induction</u>
<i>Major radiation-induced cancers</i>		
Female breast	Very high	High
Thyroid	Low	Very high
Lung (Bronchus)	Very high	Moderate
Leukemia	Moderate	Very high
Alimentary tract	High	Moderate to low
<i>Minor radiation-induced cancers</i>		
Pharynx	Low	Moderate
Liver and biliary tract	Low	Moderate
Pancreas	Moderate	Moderate
Lymphomas	Moderate	Moderate
Kidney and bladder	Moderate	Low
Brain and nervous system	Low	Low
Salivary glands	Very low	Low
Bone	Very low	Low
Skin	High	Low
<i>Sites or tissues in which magnitude of radiation-induced cancer is uncertain or has not been observed</i>		
Larynx	Moderate	Low
Nasal sinuses	Very low	Low
Ovary	Moderate	Low
Connective tissues	Very low	Low
Prostate	Very high	Absent?
Uterus and cervix	Very high	Absent?
Testis	Low	Absent?
Chronic lymphatic leukemia	Low	Absent?

Adapted from BEIR III Report, (1980).

and, in some cases, quite scarce. However, much has been learned from animal studies that bears on the basic mechanisms of radiation oncogenesis (Fry, 1984). Further, in the absence of data specifically on low-dose radiation effects in people, life-span studies in animals have yielded much information of value (Thompson and Mehaffey, 1986), even though they have been little used in promulgation of human risk estimates (BEIR III Report, 1980). The remainder of this report discusses in detail each of the factors listed in Table 1.2.

1.2.2 Basic Biological Factors and Mechanisms in Radiation Oncogenesis

Research in radiation, chemical, and viral oncogenesis has led to a recognition of the multistage nature of the process. This work also has led to an understanding of many of the factors which play a role in modifying oncogenesis. The nature of these processes and factors *in vivo* are discussed below.

1.2.2.1 Oncogenesis, a Multistage Process

It has been accepted for many years that cancer development is a multistage process that reflects a stepwise cellular evolution from normal through initiated, preneoplastic and premalignant, to neoplastic and malignant cells. During this process, fully neoplastic cells develop, meaning that such cells can proliferate without the need for an added growth stimulus; i.e., they have acquired the capability of a degree of autonomous growth (Farber, 1981). Research into these phenomena in animal systems has used both chemical (Yuspa and Poirier, 1988) and physical (Fry et al., 1982; Fry and Storer, 1987) carcinogens in a variety of organs and tissues. Three major steps generally are recognized; initiation, promotion, and progression (Farber, 1982).

According to Fry and Storer (1987), there is no evidence that the various processes involved in these major steps must be singular; i.e., they may come about in a variety of ways. Fig. 1.1 illustrates possible stages at which radiation may impact oncogenesis. The various terms and concepts are discussed below.

1.2.2.1.1 Initiation

A brief exposure to a carcinogenic agent, including ionizing radiation, induces a change in cells that is considered to be the first step in the multistage process and is referred to as "initiation" (Farber, 1982; Fry et al., 1982; Fry and Storer, 1987). This event does not produce a neoplastic cell, but induces some change that enables a cell to be differentially stimulated to produce focal proliferations. The evidence indicates a genetic basis for initiation (Yuspa and Poirier, 1988), and it is assumed that this change takes place in the genomic DNA of the affected cell for both chemical and radiation exposures. Initiation has been described as a rare event or series of events (Farber, 1982). Evidence from studies with both chemicals and radiation have suggested that this may not be the case and that initiation may be a more common event than previously recognized (Gould, 1984; Mulcahy et al., 1984; Clifton et al., 1986). Certainly, it appears to be a more common event than would be reflected by the much lower actual incidence of tumors in irradiated experimental animals (Fry et al., 1982). Radiation may cause initiation in many cells that never express the neoplastic phenotype, or that may do so only after interaction with other endogenous or exogenous factors (Fry and Storer, 1987).

Table 1.2 Factors That Influence Radiation Risk Estimates

<u>Radiation Characteristics</u>	<u>Biological Characteristics</u>	<u>Approach to Analysis</u>
Dose	Age	Dose-response models
Dose rate	Sex	Projection models
Fractionation	Genetic background	Absolute risk
Radiation quality	Special features of tissue or organ under study	Relative risk

From Fry and Storer, (1987).

Fig. 1.1 Possible pathways in multistage radiation carcinogenesis

1.2.2.1.2 Promotion

Promotion describes the process whereby the formation of neoplasms is accelerated (decreased latent period) or encouraged (increased frequency) in a tissue which has been exposed to an initiating dose of carcinogen (Farber, 1981; Yuspa and Poirier, 1988). Although a variety of biochemical and metabolic changes may accompany the action of promoting agents, cell proliferation is an essential phenomenon in promotion (Farber, 1982). A more specific definition, then, is that promotion is the process whereby initiated cells form focal proliferations--that is, clonal expansion--which, in turn, may act as precursors for subsequent steps in the oncogenic process.

Although most promoting agents are not genotoxic by themselves (Yuspa and Poirier, 1988), ionizing radiation is considered to be a "complete carcinogen," acting as both an initiating and a promoting agent, at least when sufficient doses are given (Fry *et al.*, 1982). Increasing cell proliferation by partial hepatectomy can increase the yield of liver neoplasms after irradiation, but large enough doses of radiation may abrogate the need for such extra stimuli by causing significant cell killing followed by compensatory repopulation (Brooks *et al.*, 1982).

1.2.2.1.3 Progression

Focal proliferative lesions seen after initiation and promotion may be preneoplastic or neoplastic, the latter usually benign (Yuspa and Poirier, 1988). Generally, preneoplastic lesions can undergo regression or differentiation. The number of putative preneoplastic nodules can be 10 to 100 times larger than the number of nodules that will undergo further steps in the oncogenic process (Farber, 1982). These further steps are believed to confer new properties on the promoted cell population that lead to further sequential phenotypic changes. This phenomenon is referred to as progression. Putative, non-genotoxic promoting agents do not appear to affect the preneoplastic phenotype of the cells involved or the incidence of benign or malignant neoplasms. Thus, non-genotoxic promoters do not affect neoplastic progression (Yuspa and Poirier, 1988). Repeated exposure to known genotoxic promoters, or to initiating agents, does affect progression, increasing the yield of malignant tumors. This suggests the need for further genetic changes in the process of progression. Ionizing radiation, being a genotoxic agent, would be expected to be able to act on neoplastic progression to increase malignancy and, in fact, has recently been demonstrated to do so (Jaffe *et al.*, 1987).

The ICRP (1977) described radiation oncogenesis as a "stochastic" effect; that is, one for which the probability of its occurrence, rather than its severity, is a function of dose. This specification has been widely accepted. However, in view of the data on neoplastic progression above, we might now question the nature of radiation oncogenesis as an "all or none" or stochastic phenomenon. Gould (1984) presented evidence that suggested that initiation was not "all or none" in nature. Also, the degree of malignancy of some tumors has been seen to increase with increasing dose of radiation. The latter observation was true for Harderian gland tumors as well as other tumor types (Fry and Storer, 1987). It is now clear that the dose of ionizing radiation can influence each of the steps of oncogenesis: that is, *initiation*, *promotion*, and *progression* (see Fig. 1.1).

1.2.2.2 Direct vs. Indirect (Abscopal) Effects *In Vivo*.

The human data with respect to radiation oncogenesis suggest that in order for a tissue to be at risk it must be directly exposed to the radiation, and that the risk depends primarily on the dose to the tissues in question (Storer, 1986). In contrast, data from animal studies clearly show that indirect or abscopal effects can play an important role. Total body irradiation is essential for the efficient induction of thymic lymphomas in mice, and the shielding of any portion of the body that contains significant hematopoietic potential, such as the spleen or thigh, can reduce the frequencies of lymphomas significantly (Yokoro, 1986). Ovariectomy prior to irradiation can dramatically change the incidence and spectrum of neoplasms in mice (Storer et al., 1982). Also in mice, although not in rats, mammary tumors can be produced in the mammary gland irrespective of the volume of tissue irradiated (Shellabarger et al., 1986). While non-uniform exposures may act indirectly to alter tumor expression, most of the data suggest that the primary target tissue for oncogenesis cannot be completely spared from exposure.

1.2.2.3 Age at Exposure

There is much information, both from studies of people and animals, on the effect of age at exposure on the frequency of radiation oncogenesis. Clearly, this is an important determinant in human risks (BEIR III Report, 1980). There certainly appear to be differential sensitivities of oncogenesis with respect to age. Still, the general statement can be made that for all species, susceptibility to radiation oncogenesis decreases with increasing age at the time of exposure (Storer, 1986). The dependence on age can vary for different types of neoplasms. For example, in mice, the rate of thymic lymphoma induction decreases rapidly with increasing age after 3 to 5 weeks, whereas, for myeloid leukemia, advancing age has little effect, at least up to six months of age (Yokoro, 1986).

One area of considerable controversy is that of the susceptibility of the fetus to radiation oncogenesis. A heightened sensitivity to radiation oncogenesis in the human fetus in the form of an increased risk of childhood cancers has been reported in a number of epidemiologic studies (Stewart et al., 1958; Shiono et al., 1980), but not in others (Jablon and Kato, 1970; Oppenheim et al., 1975). The lack of such an effect on childhood cancer in the atomic-bomb survivors (Jablon and Kato, 1970) has now been supplemented by recent data suggesting that prenatally exposed persons have a greater lifetime risk for cancer than persons exposed postnatally (Yoshimoto et al., 1988). The putative lack of a confirmation of such effects in animal studies has been cited by some as a reason to question the "biological validity" of this phenomenon (Monson and McMahon, 1984; Harvey et al., 1985). The statement in the BEIR III Report (1980) that, "This apparent special sensitivity of the fetus has not generally been borne out by animal studies," is misleading. As reviewed by Sikov (1981), there is considerable information from animal studies to indicate that irradiation of prenatal animals can lead to increased, as well as decreased, incidence of postnatal tumors.

Clearly, there are differences relating to species, as well as differences relating to the specific conditions and experimental procedures that were used. More recent studies have shown increased sensitivity (Covelli et al., 1984; Sasaki, 1987), or no sensitivity (Kusama and Yoshizawa, 1982) of the fetus. However, data from studies with dogs are consistent with an increased fetal

sensitivity. A life-span study of beagles after late prenatal and early postnatal whole body exposures indicates that there is both an increase in benign and malignant tumors in young dogs (Benjamin et al., 1986; 1987), as well as an increase in fatal malignancies in adult animals (Benjamin et al., 1987). Considering that it is accepted that the fetus and the newborn have a heightened sensitivity to both chemical and viral oncogenic agents (Rice, 1988), it is appropriate to expect a similar heightened susceptibility for radiation, as well.

1.2.2.4 Sex

Clearly, sex is a major determinant in the response of animals and humans to radiation-induced neoplasia and, in fact, to neoplasia in general. The most obvious differences are in a tissue like the breast where men and male animals rarely are affected by breast cancer. Likewise, radiation-induced breast cancer is restricted primarily to females (BEIR III, 1980; Fry and Storer, 1987). Aside from the other obviously sex-related tissue neoplasms, such as those of the ovary in mice, thymic lymphomas and myeloid leukemias are dramatically affected by the sex of the animal. Female mice have a much higher incidence of radiation-induced thymic lymphomas than do males, while the opposite is true of myeloid leukemias.

1.2.2.5 Latent Period

The latent period is that time between the exposure of an individual to a carcinogen, such as radiation, and the appearance of a clinical neoplasm. The latent period varies among species and is a function of the natural life span and the type of neoplasm. Latency also varies inversely with radiation dose (Upton, 1984). Such dependencies are true for a variety of animal neoplasms, although the latent period is generally shorter for leukemias than for solid tumors (Upton, 1984), as is also the case for human tumors (BEIR III Report, 1980).

An important question is whether or not radiation induces a true increase in the incidence of a neoplasm of a certain type, or an apparent increase because of a reduction in the time necessary for appearance of the neoplasm (decreased latent period). This question is important since it has been suggested that different mechanisms could be invoked for the two different effects (Fry, 1981). Many animal studies have shown increases in the incidence of specific tumors after irradiation, yet these could be misleading. If animals are not followed for their full lifetime, an apparent increase in incidence could simply be a reflection of an earlier time of appearance. This situation has been shown to be true of lung tumors in mice; that is, an advancement in time of appearance with no evidence of an overall increase in the incidence. On the other hand, an advancement in the time of death only, when extrapolated to the human situation, nonetheless has real and obvious significance. Decreasing the latent period for tumors which may occur late in life could advance their appearance enough to have them manifest prior to death due to other intercurrent causes. Essentially, their earlier appearance would be interpreted as an increase in the incidence that was recognized in that irradiated population.

1.2.2.6 Host Responses

There is no question that various host factors play a critical role in oncogenesis, radiation oncogenesis included. It is unfortunate that, aside from some of the factors already discussed, this is a topic that has received relatively little attention. Perhaps the most is known about the effects of hormones on some types of radiation-induced tumors. Much less is understood--the

general subject itself is much more controversial--about the role of the immune system in radiation oncogenesis. Relatively little is known about other *in vivo* factors that could affect responses to radiation, such as cell-cell interactions, tissue organization and microenvironments, growth factors, and the like. What is known in general is discussed below and, in greater detail, with respect to some specific organs.

1.2.2.6.1 Hormones

Hormone levels are well recognized to affect the appearance of neoplasms in a population. Perhaps the best model system studied has been the mammary gland in the rat and, to a lesser degree, in the mouse (Shellabarger et al., 1986). However, the full role of the endocrine system in this process is not understood. Prolactin is also well known to cause a dramatic increase in the incidence of radiation-induced Harderian gland neoplasms in rodents (Fry et al., 1976).

The hormonal control of the thyroid gland is important in thyroid oncogenesis by radiation (Clifton, 1986). Here too, we are just beginning to understand the mechanisms involved. Hormonal status has a critical effect on the appearance of ovarian tumors after irradiation (Watanabe et al., 1986). Finally, hormonal status, as manipulated by ovariectomy, can be shown to affect the incidence of radiation-induced hematopoietic and solid neoplasms in mice (Storer et al., 1982). The fact that radiation-induced thymic lymphoma is decreased in ovariectomized female mice certainly suggests that a prime determinant of the difference in incidence in males and females is hormonal in nature. It is noteworthy that the properties of three of the four human tissues that are identified as highly radiation sensitivity are significantly influenced by hormonal factors.

1.2.2.6.2 Immune System

The concept of immunological surveillance--that is, the idea that the immune system plays a major role as a first line of defense against the development of neoplasms (Burnet, 1971)--has undergone a pendulum swing in terms of perceived importance. At present, immune surveillance is not as popular a concept as it once was (Stutman, 1984; Fry and Storer, 1987). There is no question that people with defective or deficient immune systems can have increased risks of some types of neoplasms, most notably lymphomas (Stutman, 1981; Purtilo and Sakamoto, 1984), but the case for a true surveillance system, even in immunodeficient animals, is controversial (Stutman, 1984).

Radiation has long been recognized to be a powerful immunosuppressive agent (Anderson and Warner, 1976). However, this subject is complex and different lymphocyte subsets have varying radiation sensitivities (Anderson et al., 1986). It is of particular note that very low-dose radiation can actually augment the immune response, presumably because of the uniquely high sensitivity of the suppressor T cell which modulates the immune response (Doria et al., 1982; Anderson et al., 1986). While it is logical to consider that the immunosuppressive effects of radiation could play a role in radiation oncogenesis, this possibility has not been clearly established (Fry and Storer, 1987).

In reference to the preceding, examples of contradictory data include the results of studies of the effect of neonatal thymectomy on plutonium-induced lung

cancers in rats (Nolibe *et al.*, 1981). Unirradiated, thymectomized (immune deficient) rats had an increased frequency of extra-pulmonary neoplasms. After inhalation of plutonium, irradiated rats had an increase in pulmonary tumors, but this effect was not increased further in the thymectomized, immune deficient animals. On the other hand, the malignancy of the radiation-induced tumors, as evidenced by tumor size, invasiveness, and number of metastases, was increased in the thymectomized animals. So, in the face of a general increase in neoplasms in unirradiated immunodeficient rats, thymectomy did not affect the frequency of radiation-induced tumors even though it did increase their malignancy.

Another example of a study that is paradoxical is the observation that low-dose radiation actually enhanced an antitumor response (Anderson *et al.*, 1982). Low dose (5-25 cGy), whole-body exposures of mice prior to the transplantation of sarcoma cells resulted in a lower incidence and smaller tumors in irradiated recipients. The radiosensitive T-suppressor-cell was implicated in this phenomenon, possibly through augmentation of immune responses by T-suppressor-cell killing.

The overall role of the immune system in radiation oncogenesis, especially due to low doses, remains to be more fully elucidated.

1.2.2.7 Interaction of Radiation with Other Agents

Any combination of agents, chemical or physical, can have varying effects with respect to oncogenesis. These may be additive, synergistic, or antagonistic. Because this subject has been extensively reviewed (Fry and Ullrich, 1986; NCRP, 1989), and because of its inherent complexity, only the more general dimensions of the topic are described.

Radiation is a complete carcinogen in that a single exposure can induce neoplasia. On the other hand, low doses may initiate cells and these may have a low probability for expression unless they are exposed to some other, second agent. Further, radiation may serve as the second event in oncogenesis initiated by some other agent. Such possibilities are obviously dependent on the sequence, frequency, timing and total exposures of these various agents.

In rats, exposure to radon-daughter products and cigarette smoke resulted in an increased risk for lung cancer if the cigarette smoke followed the radon-daughter exposures, suggesting a promoting effect of the smoking (BEIR IV, 1988). Other studies have reported the interaction of ionizing radiation with chemicals such as urethane and benzo(a)pyrene in increasing the induction of lung tumors in animals (Fry and Ullrich, 1986).

Both enhancement and reduction of skin oncogenesis has been reported for the interaction of ionizing radiation with various chemicals, although most of these studies have involved the evaluation of secondary chemical effects after initiation by radiation (Fry and Ullrich, 1986). A few older (Fry and Ullrich, 1986) and more recent (Jaffe *et al.*, 1987) studies do indicate that radiation can act to effect promotion/progression of chemically initiated skin tumors.

Both mammary oncogenesis and leukemogenesis have been affected, either increased or decreased, by the interaction of radiation with chemicals, depending on the doses used and the sequence of events involved (Fry and Ullrich, 1986).

The importance of viral agents in radiation oncogenesis is equivocal. The best known model is thymic lymphoma in the mouse. The role of the so-called radiation leukemia viruses is discussed in reference to the hematopoietic system. The other example of note is the induction of bone tumors in mice. Radiation-induced osteosarcomas in mice have been found to be associated with the presence of viruses which can cause osteosarcomas (Finkel et al., 1976; Goldman, 1986). These viruses would appear to play a role as evidenced by the facts that vaccination can delay the onset of radiation-induced bone tumors, and that injection of active virus into irradiated animals can increase their lifetime incidence of bone tumors (Reif and Triest, 1983).

1.3 DOSE AS A FACTOR IN RADIATION ONCOGENESIS

The problem of defining dose-response relationships is a central one for risk estimation. The nature of the dose-response curves has been discussed extensively, and these concepts have undergone continuous revision (BEIR I Report, 1972; BEIR III Report, 1980; NCRP, 1980) and have been revised once again (BEIR V Report, 1990). The information upon which the accepted dose-response curves is based is related to the primary events in oncogenesis (Fry and Storer, 1987). For many relatively simple biological endpoints, such as the induction of chromosome aberrations and other *in vitro* effects, the dose-response models in use fit well. Human data on radiation oncogenesis have large errors associated with them and, consequently, several models may appear to fit the data (Storer, 1986). Most of the data, from both human and animal studies, that have been used to construct dose-response curves were derived from relatively high radiation doses. In fact, the *in vivo* data on oncogenesis at low doses, <10 cGy, is quite scant, especially for low-LET radiations. Thus, modelling at low doses has been based upon extrapolations of effects after high doses.

1.3.1 Dose Response Models in Humans

There have been few large epidemiological studies of the effects of radiation on humans where the exposed populations have been followed until the end of life. As a result, there is substantial uncertainty in projection of currently available health risk data for such populations to the end of life, and certain assumptions must be made in order to make such projections. In addition, much of the available epidemiological data, which has produced statistically significant results, is associated with populations exposed to relatively large doses of radiation and usually at high dose rates. As a result, there is also great uncertainty in the dose response curves for the end points of interest (e.g., cancer and hereditary disorders). The various biological dose response models (linear, linear-quadratic, and quadratic models) will also be briefly presented and evaluated in light of current knowledge.

Generally speaking, neither human nor animal response data provide adequate information to statistically establish the shape of the biological response curve at low doses, because the sample sizes required to unequivocally do so are too large to be practical. The size of the required sample population is approximately inversely proportional to the square of the cancer excess. For example, if 1,000 animals in the exposed and control groups are required to provide adequate statistical power at 100 rads, about 10,000,000 in each group would be required at 1 rad, and about a billion in each group would be required at 0.1 rad (BEIR III Report, 1980, p. 140).

Although there are several models which purport to describe the biological response from exposure to radiation, at present only three have achieved some measure of acceptance based on theory and observed responses of human populations, and of whole animals and autonomous cell lines grown in culture. All three of the models are derivable from a generalized model having both linear and quadratic terms, and all assume no threshold for cancer risk (even though none are statistically detectable below about 10 cGy in humans). The discussions which follow will briefly describe these models, which are shown graphically in Fig. 1.2, taken from BEIR III, 1980.

1.3.1.1 Linear-Quadratic Response Model

Because neoplasia or mutagenesis results from changes in individual cells, biological response models are derived from basic theories of cellular damage from ionizing radiation of various qualities. The linear-quadratic dose dependency frequently has been found to be adequate to fit observed responses of autonomous cells exposed to low-LET radiation such as X-rays, γ -rays, and β -particles. Data from studies of cellular responses generally can be fitted with an equation of the following type:

$$E = a + bD + cD^2$$

where a is the zero-dose incidence (i.e., spontaneous rate), D is dose, and b and c are empirically derived coefficients. Another consideration would be to account for cell killing (see Section 3.2), but cell killing usually is not relevant at low doses and low dose rates.

This model almost certainly oversimplifies the real situation in cells (e.g., no accounting is made of: the variation in the sensitivity of cells as they proceed through the cell cycle; time-dependent events such as cell repair/misrepair; or the effects of cell killing at high doses on the likelihood of detecting cell transformation). However, for fitting human epidemiological data, the linear-quadratic model is believed to be adequate in the instance of leukemia (BEIR V Report, 1990).

1.3.1.2 Linear Response Model

The linear response model has been used to fit human data for tumorigenesis typically following high doses, generally at high dose rates (e.g., cancer incidence among the Japanese bomb survivors). The model is based on the assumption that there is no threshold dose for radiation health effects, so a straight line can be drawn from the zero dose incidence through the effects observed at high doses. Assuming linearity means that the quadratic term in the general model is too small to be important even at doses approaching lethal levels. While this assumption ignores animal and cellular data to the contrary, the statistical tests on human data have lacked the power to reject either the linear or the linear-quadratic models. Given this situation, and the greater ease in using the linear model to calculate risks at different doses, the simpler model has been favored. The coefficient of the term aD is the slope of the line fitted to the lifetime projected cancer risks that are available largely from high-dose, human data.

In the Report of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR Report, 1989) the linear-response model was adopted;

Figure 1.2 Alternative dose-response curve for radiation oncogenesis. From BEIR III Report, (1980).

however, dose-rate effectiveness factors (DREF's) for use at low dose-rates, less than 10 cGy per year of a low-LET radiation, were also recommended. The factors selected by the National Radiological Protection Board are 2 for breast cancer and 3 for all other cancers (NRPB Report, 1988, pp. 24-25 and 60-62). This approach is similar to that employed in the Reactor Safety Study (USNRC, 1975). The latest report of the BEIR Committee (BEIR V Report, 1990) concluded that, in addition to leukemias, data for all other cancers were fitted best by a linear response model.

1.3.1.3 Quadratic Response Model

The quadratic response model was considered by the BEIR III Committee to fit reasonably the available high-dose data, and to provide a lower bound to the envelope of risks--relative to a linear-quadratic dependence--that it was developing for Federal guidance (BEIR III Report, 1980, p. 142).

Acceptance of the quadratic model as a reasonable description of the risk to radiation exposure appears questionable, since, in a classical sense, it implies that single hits are unimportant in production of cancer, and all the observed effects can be explained in terms of double-tracks. While that may be true for existing high-dose, low-LET, human-risk data, the use of such models to extrapolate to the risk of low-dose, low-dose-rate exposures is scientifically questionable.

1.3.2 Dose Factors in Radiation Oncogenesis in Animals

Experimental studies with animals have been addressed to many questions that usually cannot be answered from human data. Although the shape of the dose-response curve at low doses remains questionable, much has been learned with animals relative to the effects of dose rate, radiation quality, and dose localization.

1.3.2.1 Dose

One problem with various dose-response models is the suspicion that various factors, which may influence the dose-response curves, may not apply uniformly over a wide range of doses (Fry and Storer, 1987). As noted, oncogenesis appears to be a complex, multistage process and the final expression of tumor incidence may be a reflection of more than the initial biophysical and molecular events in the target cells. The mechanisms that play a role in the induction of different types of tumors may be diverse enough that a single dose-response model may not adequately fit them all. For example, it is not known whether or not there are essential differences in mechanisms of induction between tissues of high sensitivity, and therefore capable of being induced by low doses (breast, thyroid, bone marrow) vs. those tissues with low sensitivity that can be induced only after substantial exposures. Such differences could reflect different mechanisms of initiation or different influences of the host on the later stages of the oncogenesis process. Thresholds may exist for some host responses, such as induced hormonal imbalances or immune suppression, and possibly not for others, such as cell killing.

Probably because of factors such as those mentioned above, a single model for radiation oncogenesis will probably not fit all animal data. Fry (1981) cites experimental data which show that almost any type of dose-response (linear, no-threshold; non-linear, no-threshold; or non-linear, threshold) can be seen

depending on the organ system involved and the species used. In general, the frequency of radiation-induced neoplasia increases with increasing dose. Frequently there is a maximum followed by either a decline or a plateau that is probably attributable to the killing of target cells in some instances, e.g., at high doses and dose rates of low-LET radiation.

1.3.2.2 Radiation Quality

It is clear that a primary determinant of the shape of dose-response curves is radiation quality. For low-LET radiation, the situation appears to be complex. A variety of experiments have yielded conflicting data, with some showing a linear response for all tumor types (Thomson and Grahn, 1988; Maisin *et al.*, 1983a; Maisin *et al.*, 1983b), especially at low doses. Other data fit both linear and linear-quadratic responses, depending on the sexes and strains of the animals that were used and whether or not all tumors were grouped together or evaluated as individual types (Storer *et al.*, 1979; Ullrich, 1980; Ullrich *et al.*, 1987). Further complicating the picture is the confirmed finding that some tumor types, such as those of the ovary, appear to show a threshold response (Ullrich, 1980), an observation that appears to be at odds with the model generally used (BEIR III Report, 1980).

Data for high-LET radiation are quite different from those for low-LET but, once again, the simple linear model does not work in all instances. The initial slopes are steeper than those for low-LET radiations (Fry and Storer, 1987). At doses that vary, but may be as low as 10-20 cGy, the curves may bend downward from a linear response and even become flat despite increasing dose (Fig. 1.3). As noted above, this plateau phenomenon is a general characteristic of radiation oncogenesis and it occurs at high doses with low-LET radiation and at low doses with high-LET exposures. Increasing the LET by using various heavy ions (Fig. 1.4) clearly increases the effectiveness of tumorigenesis relative to low-LET radiation.

1.3.2.3 Dose Rate, Fractionation, and Protraction

As has been pointed out by Ullrich (1980), instantaneous dose rate, fractionation, and protraction can produce varied, dose-response dependencies. Total dose, dose rate, fraction interval, and dose per fraction all are important in interpreting experimental results. Protraction, that is, spreading a dose out over a longer period of time, can be important in that it can span a range of cell stages and host ages that have differing sensitivities to oncogenesis. While protraction has been used in experiments with external exposures (Grahn and Fritz, 1986), it is particularly important in studies using internally deposited radionuclides (Wrenn *et al.*, 1986a, 1986b; Goldman *et al.*, 1986; Thompson *et al.*, 1986; McClellan *et al.*, 1986).

Decreasing dose rate clearly decreases the effectiveness of oncogenesis by low-LET radiation, although the degree differs for different types of tumors (Ullrich, 1980; Fry, 1981). Studies using Harderian-gland tumors, thymic lymphomas, lung tumors, and mammary-gland tumors have also demonstrated that changing the dose rate can change the shape of the dose-response curve. For all of these, lowering the dose rate caused a change from a linear-quadratic to a linear dose response (Ullrich, 1980; Ullrich *et al.*, 1987). For low-LET radiation, the effect of fractionation can be dependent on the number and size of the fractions. For example, for the same total dose, a smaller number of fractions can be more effective than a larger number. With some tumors, such as

Figure 1.3. Age-adjusted incidence of thymic lymphomas and Harderian gland neoplasms as a function of fission neutron dose (From Fry and Storer, 1987).

Figure 1.4. Harderian gland neoplasms in mice after heavy ion and gamma irradiation. Dose-average LET values estimated as : ^4He , 1-2 keV/ μm ; ^{20}Ne , 150 keV/ μm ; ^{56}Fe , 190 keV/ μm ; and ^{40}Ar , 650 keV/ μm (From Fry and Storer 1987).

thymic lymphomas and reticulum-cell sarcomas in mice, dose fractionation or low-dose rate irradiation can increase tumorigenic potential (Fry and Storer, 1987). Such anomalous findings might reflect changes in host responses, as well as direct radiation effects on the target cells.

For high-LET radiation, lowering the dose rate or protracting the dose by multi-fractionation did not reduce tumorigenesis and, in fact, in some instances enhanced it (Thomson et al., 1981; Maisin et al., 1983b; Storer et al., 1979; Fry and Storer, 1987). Ullrich (1984) demonstrated that changing the dose rate or fractionation for neutrons could have differential effects on different tumor types, that is, lowered dose rate enhanced mammary oncogenesis but reduced ovarian neoplasm incidence. From this, it was suggested that different mechanisms of induction might be involved for different tissues.

Protraction of the dose can be important in oncogenesis as illustrated by studies of continuous γ -ray exposures of dogs. The induction of myeloproliferative disorders and myeloid leukemias was dependent on dose rates low enough to allow dogs to accumulate sufficiently high doses (Fritz et al., 1986). With exposures of 5 to 10 cGy/day, and total doses of 20 Gy, 50% of the dogs developed myeloproliferative syndromes. Dose rate and dose protraction have also been shown to be critical factors in pulmonary oncogenesis from inhaled internal emitters with different dose patterns leading to differences in incidence and type of neoplasms (Hahn et al., 1986).

1.3.2.4 Dose Localization

Localization of the dose to specific portions of the body or to specific tissues is obvious in its potential importance, assuming that there must be direct exposure of a tissue to result in oncogenesis. Beyond this, whole body exposure could affect organs or tissues other than the one in which the tumors are observed. Localization of the dose is a more critical factor when dealing with internally deposited radionuclides (Boecker et al., 1986; Book et al., 1986; Wrenn et al., 1986b; Taylor et al., 1986; Miller et al., 1986) and range of radioactive particles such as α -particles and the dose to the target cells must be taken into account in an evaluation of dose-response dependencies.

1.4 MODELS FOR PROJECTING LIFETIME RISKS OF CANCER IN HUMANS

Once a dose response curve has been developed based on the observed mortality, it is necessary to develop a model for projecting the future effects which might occur as a result of past exposures to living populations of people. In this section, the two prominent models used to project current data, the relative risk and absolute risk models, will be briefly presented. Risk projections require a knowledge of parameters such as: the latent period (i.e., the time from exposure to the commencement of increases in cancer mortality rates); the plateau period (i.e., the time during which risk of cancer is increased); the relationship between the spontaneous risk and the excess risk; the age distribution of the exposed population and the age-specific mortality rate from all causes including the types of cancers under evaluation; the effect of age at the time of exposure; the effect of sex; and possibly other factors (e.g., cigarette smoking, etc.).

The major difference between the relative risk (RR) and the absolute risk (AR) projections is that the RR accounts for the age-specific susceptibility to

cancer. Since the RR model and the AR model both utilize the same existing data, both models produce numerically consistent results for those data when calculated to the present time.

These models are not true biological models in the sense that they are derivable from theories of carcinogenesis. Rather, they are simply mathematical models used to fit observed epidemiological data. A determination of which, if either, of these models is more appropriate for future projections can only be based on how well they fit the observed data. And, of course, the observed database at this time is still growing as the exposed populations continue to age.

1.4.1 Relative Risk (RR) Model

The relative risk (RR) model is a multiplicative risk model in which the ratio of the excess cancer in an exposed population to a comparable unexposed population is assumed to remain constant after an appropriate latent period following a given dose. Therefore, the projected estimates of future cancer mortality are multiples of the natural cancer risk in the exposed population. That is,

$$R(\text{exposed}) = RR \times R(\text{unexposed}, t)$$

where RR is constant for all times t greater than the latent period.

Because the baseline age-specific cancer rates in an unexposed population increase rapidly with advancing age, the projected (i.e., the calculated) cancer rates in the exposed population will also increase rapidly with age following exposure (BEIR III, 1980, pp. 30-31). This relationship is shown in Fig. 1.5, taken from the BEIR III Report (1980).

When the most current data were examined, this model was found to be appropriate for projecting future risk for most tumors (BEIR V Report, 1990).

1.4.2 Absolute Risk (AR) Model

The absolute risk model (AR) is an additive model in which the expression of cancer is independent of the natural age-specific cancer following exposure. After an appropriate latent period, where no excess cancers are detectable in an exposed population, this model assumes that the cancer induction rate is constant per unit of dose (BEIR III Report, 1980, p. 30). For a given exposure, the rate is assumed constant and additive to the spontaneous cancer rate for an additional period of time following the latent period (the so-called plateau). That is,

$$P(\text{exposed}, t) = AR + R(\text{unexposed}, t)$$

where AR is the absolute excess risk for all times t greater than the latent period.

This model is shown in Figure 1.5. In reports prior to the latest BEIR Committee Report (BEIR V Report, 1990), this model was felt to best represent the risk of radiogenic leukemia, and was also thought to be appropriate for projections of cancer of the thyroid, bone, and liver. See Appendix A for more details.

Figure 1.5 Radiation-induced cancer incidence superimposed on spontaneous incidence by age. Absolute and relative risk models. From BEIR III Report, (1980).

1.5 HUMAN RISKS OF RADIATION ONCOGENESIS

This section will focus on recent reviews, studies, and analyses of the human epidemiological data on cancer risks among exposed human populations. Risk coefficients for individual organs are presented along with projected life-time risks of several populations of people who were exposed to radiation as a result of the atomic bomb explosions in Japan during World War II, or as a result of various medical treatments.

There have been few, if any, large epidemiological studies of the effects of radiation on humans where the exposed populations have been followed until the end of life. As a result, there remain substantial uncertainty on the life-long risks of cancer. Since the BEIR III Report (1980), a substantial revision of the dosimetry for the exposed Hiroshima and Nagasaki populations has been completed. In addition, new data from Japanese survivors and other exposed populations have continued to accumulate. This review will focus on the results of current reevaluations of the health risks to those populations on an organ-by-organ basis.

The task of comparing the results of several recent studies is complicated by the fact that the risk data from the Japanese A-bomb survivors has just been revised as a result of a reevaluation of the bomb dosimetry. The new bomb dosimetry system, DS86, was initially developed by SAIC under contract to the Department of Energy (Kaul, 1983). The final dosimetry report was published in 1987 (Roesch, 1987). The principal changes resulting from the reassessed dosimetry follow.

- (1) In Nagasaki, the γ -ray kerma (kinetic energy released in matter; approximately the first collision dose) for DS86 was about 10 to 30 percent less than for the 1965 tentative dosimetry system (T65D), while the neutron kerma was about one-half to one-third of the T65D estimate;
- (2) In Hiroshima, the γ -ray kerma for DS86 was about 2 to 3.5 times larger (depending on ground range) than the T65D value, while the neutron kerma was only about 10 percent of the T65D value.
- (3) A major factor which kept the risk coefficients from rising even further was the reduction in the gamma-ray transmission factor in Japanese houses; 0.53 for prompt gamma and 0.46 for delayed gamma at 1,500 m vs. an assumed T65D factor of 0.9. The neutron transmission factor was not a great deal different for DS86 (0.38) compared with the T65D value (0.32).
- (4) In addition, the organ dose estimates were considerably more sophisticated than T65D, and resulted in a much higher average organ transmission factor, compensating in part for the reduced gamma-ray transmission factors for houses.

Subsequently, Preston and Pierce (1987) provided new estimates of risk based not on kerma, but upon calculated organ doses. The authors warned that it would be erroneous to base risk estimates on kerma only, since organ doses and dose equivalents were quite different. As a result, the authors concluded that there was relatively little difference between the DS86 and T65D dosimetric value for total organ dose. Nevertheless, due largely to the reduction in the neutron and gamma dose estimates, there is now relatively little difference in risk

estimates (on a per rem basis) between Hiroshima and Nagasaki, resolving a long-standing point of contention about the actual doses in the cities.

Before discussing radiogenic cancer risks to individual organs, it would be wise to first discuss the overall risks associated with total body (or nearly so) irradiation. For this assessment, the major source of information must come from the Hiroshima and Nagasaki A-bomb survivors. The most recent information comes from four major reviews: the Radiation Effects Research Foundation in Japan (RERF; formerly the Atomic Bomb Casualty Commission established in 1977 by the U.S. National Academy of Sciences), the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the U.K. National Radiation Protection Board (NRPB), and most recently, the U.S. National Research Council's Committee on the Biological Effects of Ionizing Radiation (BEIR).

The most recent RERF report (Shimizu *et al.*, 1988) provides an estimate of the overall increase in the risk of leukemia and total solid tumors based on the DS86 dosimetry. The report indicates that the best fit for the solid tumor data now is a linear dose response model, with a relative risk projection to the end of life for the populations under study. The relative and absolute risks of leukemia are discussed in Section 1.6.1. The relative risk of all cancers except leukemia following 100 cGy of low LET radiation to the whole body (the neutron contribution to dose is now considered negligible), at a high dose rate was estimated to be 1.41 (1.32-1.51, 90% confidence interval (CI)). The excess (absolute) risk estimate is 10.13 (7.96-12.44, 90% CI) per million person-yr-cGy.* The total cancer mortality risks were 7.62 at 100 cGy (relative risk), and 13.1 per million person-yr-cGy, respectively. Estimates of lifetime risks by RERF were still in preparation at the time of this review. Preliminary results by Shimizu *et al.*, (1988, Table 19) indicated that the magnitude of the change would be an increase in the leukemia risk of about a factor of 2.2, BEIR III Report (1980) linear model, and a factor of about 3.2, BEIR III Report (1980) linear-quadratic model, both for doses below 200 cGy. The increase in the risk of solid tumors would be about 2.7, BEIR III Report (1980) linear model, and about 5.5, BEIR III Report (1980) linear-quadratic model, both for doses under 200 cGy.

The UNSCEAR Committee (1988, Table 69) prepared independent estimates of the relative and absolute risks of leukemia and solid tumors. The details on the leukemia risk are presented in Section 1.7.1. The maximum lifetime risk of all cancers except leukemia and the maximum total lifetime cancer risk of whole body irradiation to 1,000 people each receiving 100 rads were as follows:

Relative Risk (solid tumors): 61 (48-75, 90% CI) per 100,000 person-cGy
(total cancers): 71.2 per 100,000 person-cGy

Absolute Risk (solid tumors): 36 (28-44, 90% CI) per 100,000 person-cGy
(total cancers): 45.3 per 100,000 person-cGy

Thus, the projected lifetime risk of cancer, using the recommended absolute risk model for leukemia, and the relative risk model for all other cancers would be

*Excess risk here is synonymous with absolute risk; it is the difference between the number of cancers in an exposed group minus the difference in an unexposed (control) group divided by the person-years at risk and the average dose to the population at risk (i.e., person-yr-cGy).

about 700 cancer deaths per million person-rem (assuming an effective RBE of 1 for the bomb survivors, since the reassessed neutron doses were too small to effect the results for an assumed RBE of 1 to 10. That total is about 5 times greater than the value recommended by the BEIR III Committee (BEIR III Report, 1980). In the Report of UNSCEAR (1988), it was recognized that data from both the ankylosing spondylitis patients and from animal studies clearly showed a reduction in cancer rates when the dose was protracted over periods of time. However, they were unable to agree on a dose-rate effectiveness factor (DREF) for use with populations receiving doses at low dose-rates and low doses. Although the Committee concluded that the DREF should be in the range of 2 to 10, they put off the decision on specific DREFs for another day. Unless the DREF's ultimately recommended are in the range of 2 to 3, it is possible that the new risk estimates will have major repercussions on commercial and military uses of nuclear materials if incorporated into Federal regulations.

The National Radiological Protection Board (NRPB Report, 1988) also prepared its own evaluation of the Japanese bomb survivor data using the DS86 dosimetry results, as well as what they considered useful data from other populations (e.g., post-partum mastitis patients for risk of breast cancer, radium dial painters for bone cancer, and thorotrast patients for liver cancer). The NRPB risk estimates were projected for the U.K. populations of England and Wales, DREFs of 2 for breast cancer and 3 for all other cancers. Therefore, these estimates may represent more reasonably the likely cancer risk to U.S. workers and the general population for low doses delivered at low dose rates. The NRPB estimated the lifetime risks using linear dose response and an absolute risk model with a 40-yr plateau for leukemia, and a linear, relative risk model for all other (solid) cancers. To provide what they considered a reasonable lower bound to cancer risk, the NRPB also calculated the risk to date (i.e. no lifetime projection), using the same DREFs as before to estimate risks from low-dose-rate exposure. The projected lifetime risk of all cancers except leukemia was 420 per million person-rads, and the lifetime risk of all cancers combined was 450 per million person-rads. That value is about 2 times greater than the estimate from the BEIR III Committee's linear-quadratic response model, but not greatly different from that Committee's relative risk, linear response model.

The most recent estimates of the risks of radiogenic cancer (BEIR V Report, 1990) is the most complete and definitive since the BEIR III Report (1980). Considerable controversy is likely to remain and the report may be viewed by some as overly conservative, i.e., possibly overestimating risks. However, it is unlikely that any suggested modifications to the conclusions (e.g., use of a Dose Reduction Effectiveness Factor (DREF) in the range of 2 to 3 to account for the lower risk expected from low-dose, low dose-rate radiation as opposed to the high-dose, high dose-rate based data of the A-Bomb survivors Life-Span Study (LSS), ankylosing spondylitis patients study (ASS), and other studies) will reduce the risk estimates by more than a factor of about two.

As already noted, there are now new data regarding total incidence and mortality among the A-bomb survivors. The revised estimates of the neutron and γ -ray doses indicated that the earlier estimates, T65D, were too high as well. The BEIR V Committee reviewed the new data from other study cohorts, but relied most heavily on the A-bomb LSS cohorts for its estimates of risks by site as well as overall cancer risks.

Because cancer risks are dependent on the age at exposure, the BEIR V Committee based its estimates of lifetime risk on the observed risk for each age cohort throughout their lives. Unfortunately, the numbers of observed cancers by site were often small (or zero), which made estimates at these sites impossible. The Committee focused on estimating the risks of the major cancers; that is, the risks of leukemia, the breast, the thyroid, the respiratory tract, and the digestive system. All other cancers were lumped together as a group.

The BEIR V Committee concluded that the risk of leukemia risk was fitted best by a linear-quadratic dose response formula and a relative risk projection, whereas the data for all other cancers were fitted best by a linear-response model and a relative risk projection. In the BEIR V Report (1990), a DREF of 2 is incorporated for leukemia into the risk model, but no DREF factors were used for other cancers. This step represented a significant departure from prior BEIR, NCRP, ICRP and UNSCEAR modeling. The Committee's estimates are summarized in Table 1.3, and more detail is provided in Appendix A to this report. For general population use, the "Average" column is probably the most useful, and indicates that the Committee's modeling would predict about 695 nonleukemia deaths and 95 leukemia deaths per million person-rem (based on the 1980 U.S. population), for a total of 790 deaths per million person-rem. Similarly, the estimated risk for a radiation worker population (composed primarily of males) would be about 520 nonleukemia and 83 leukemia deaths per million person-rem, for a total risk of about 600 deaths per million person-rem (the reduction is primarily due to deletion of the higher exposure risks to children and early teens). Risks to female workers would be about seven percent higher (due primarily to higher nonleukemia risks, such as breast cancer).

It should be noted, however, that the risk per person-cSv is approximately the same for both the single and continuous exposure scenarios for the general population. While some reduction in risk might have been expected for the continuous exposure scenario, the BEIR V Committee pointed out that the linear-quadratic model has an implicit dose-rate effect since the quadratic component "vanishes" at low doses. The Committee concluded that no further corrections were required for low-dose, low-dose-rate exposures such as 0.1 cSv/yr or an instantaneous dose of <10 cSv.

1.5.1 Leukemia

At present, there are few types of leukemias which have been linked to radiation exposure; most radiogenic leukemias are acute lymphocytic leukemia (ALL). The primary populations under study are Japanese bomb survivors and ankylosing spondylitis patients. Risk of leukemia among bomb survivors has been recently reviewed by several different groups, including RERF (Shimizu et al., 1988), UNSCEAR (1988), NRPB (1988), and most recently, BEIR V (1990). All four have provided recent independent estimates of the risks utilizing the new Hiroshima and Nagasaki dosimetry (DS86). As a result, summaries of risk estimates will be weighted heavily toward those studies.

The most recent RERF report (Shimizu et al., 1988) examined the cancer mortality data (1950-1985) using the DS86 dose estimates, focusing on the appearance in time of radiation-induced cancer and the effects of such risk modifiers as age at exposure, age at time of death, sex, city, and carcinogens other than radiation (e.g., smoking). They concluded that:

Table 1.3 Excess Cancer Mortality per 100,000 Exposed Persons^a

	Male	Female	Average ^b
Nonleukemia			
Single 10 cSv Dose	660 (420-1,040)	730 (550-1020)	695 (485-1,030)
Continuous (0.1 cSv/yr, Lifetime)	450 (320-830)	540 (430-800)	495 (375-815)
Contin. Lifetime (1 cSv/yr; Ages 18-65)	2,480 (1,670-4,560)	2,760 (2,120-4,190)	2,620 (1,895-4,375)
Leukemia			
Single 10 cSv Dose	110 (50-280)	80 (30-190)	95 (40-235)
Continuous (0.1 cSv/yr, Lifetime)	70 (20-260)	60 (20-200)	65 (20-230)
Contin. Lifetime (1 cSv/yr; Ages 18-65)	400 (130-1,160)	310 (110-910)	355 (120-1,035)

^aRanges represent 90% confidence limits [Table 4-2, BEIR V Report, (1990)].

^bAssumes equal numbers of males and females in the exposed population.

- 1) The excess leukemia rate continued to decline with time, but that is was still slightly elevated in Hiroshima through 1985;
- 2) For leukemia, the linear dose-response model fitted well (except for high doses) and, based on the new dosimetry, yielded risk estimates that were about twice as great as the linear-quadratic model in the BEIR III Report (1980);
- 3) When adjusted for smoking, leukemia risks were higher for females,
- 4) For children 0-10 years of age, the latent period was unaffected by the total dose;
- 5) The excess risk of leukemia varied over time (not observed for other cancers);
- 6) The excess risk of leukemia did not continue to increase, among those greater than 10 years of age when exposed, in a manner consistent with the relative-risk model; and
- 7) At 100 cGy, for leukemia the relative risk, the excess deaths (absolute risk), and the percent attributable risk per million person-year-cGy (all at 90% confidence intervals) were estimated to be 6.21 (4.83-8.12), 2.94 (2.43-3.69) and 58.6% (48.4-69.5), respectively.

The excess leukemia risks for the relative and absolute models, based on absorbed dose by age at exposure and by sex, are summarized in Table 1.4.

The most recent UNSCEAR Report (1988), although using the T65D dosimetry (which underestimates risks), nevertheless stated results similar to those reported by Shimizu *et al.*, (1988) based upon the DS86 dosimetry; that is, relative risks at >100 cGy was reported to be 9.38 (7.0-12.6), and excess risk for the same group was reported to be 3.95 (3.04-4.86) per 10,000 person-years (PY). Somewhat unsettling was the comparison with leukemia risk observed among patients with ankylosing spondylitis who were treated with X-rays in the United Kingdom during 1935-1954 (BEIR III Report, 1980). Spinal exposures for the typical 10-treatment course averaged about 542 R, and some patients were given as many as four courses of the treatment (subsequent courses averaged about 346 R).

The UNSCEAR Committee concluded that the relative risk among these patients was about 4.79 (3.4-6.6). For these patients, the excess number of leukemias was about 1.96 (1.24-2.88) per 10,000 PY, that is, substantially lower than that observed among the Japanese survivors of the A-bombs. The excess among the Japanese survivors was attributed to the lower doses and lesser degrees of cell killing as well as to the larger amount of bone marrow exposed.

The UNSCEAR Committee noted a similar, lower-than-expected rate of leukemia for women treated with external X-rays, or with intra-cavity radium sources, for cervical carcinoma. (A total of 180,000 women, from eight different nations, received 20-70 Gy over a 4-8 week period.) As with the spondylitic patients, the relative risks were lower than expected; about 1.14 (1.00-1.45, 95% CI). The absolute risk was only 0.10 (0.00-0.31, 95% CI) per million person-year-cGy. Although steps were taken to exclude the occurrence of early fatalities from other causes, which would have precluded the development of leukemia, by studying

Table 1.4 Excess Leukemia Risk for Relative (RR) and Absolute (AR) Risk Models Based on DS86 Absorbed Dose by Age at Exposure and by Sex^a

Risk Model	Sex	Age at Exposure				
		<10	10-19	20-29	30-39	40+
RR	M	17.9	4.8	5.9	4.5	3.9
	F	18.8	5.0	6.2	4.7	4.0
AR	M	3.46	1.79	3.87	5.72	4.22
	F	2.71	0.92	2.24	1.79	2.88

^a100 cSv dose for the RR, and one million person-cSv for the AR assuming a neutron RBE of 10.

only those women who survived for 10 or more years, it is still conceivable that competing causes of death among cancer patients could have contributed to the fewer cases of leukemia that were observed.

However, the use of cancer data from people treated for malignant disease can involve other problems when attempting to relate the frequencies observed with those for the general population. First, some people have predispositions to cancer (e.g., colorectal and breast cancer), and second, many cancer patients treated with radiation are also treated with chemotherapy (e.g., alkylating agents known to cause DNA damage). Additional confusion sometimes results because the medical community often lumps leukemias together even though some leukemias (e.g., acute non-lymphocytic leukemia) are not believed to be caused by radiation exposure. (Further discussion of treatment-induced neoplasia among cancer patients is contained in Appendix A.)

In the UNSCEAR Report (1988), Table 69 provides the Committee's estimates of lifetime excess leukemia risks for 1,000 persons each receiving a 100 rad dose at high dose rate: 9.7 (7.1-13, 90% CI) per 100,000 person-rads (relative risk) and 9.3 (7.7-11, 90% CI) per 100,000 person-rads (absolute risk).

The report of the NRPB (1988) used the age-specific risk coefficients from the paper by Shimizu et al., (1988) to calculate the lifetime risks of leukemia for the populations of England and Wales based on the 1985 U.K. life table. The NRPB concluded that the lifetime absolute risk of leukemia from exposure to high dose-rate low-LET radiation would be about 84 per million-person-rem; for low dose rate situations, the NRPB would divide that value by a DREF of 3, giving about 28 leukemia deaths per million person-rem. The NRPB compared their value with that suggested by the ICRP (1977) of 20 deaths per million person-rem for occupational exposure (based largely on use of the out-dated T65D dosimetry and early cancer mortality data for the Japanese survivors). Given that the ICRP guidance is for adults only (age >15), and the NRPB value applies to an entire population reflecting all age groups (including children and teens), these results are in good agreement.

The BEIR V Committee also depended largely on the report of Shimizu et al., (1988) for data on the A-bomb survivors. Due to limitations on the observed cases of various leukemias, the Committee chose to simplify their risk model by combining all hematologic types of leukemias except chronic lymphocytic leukemia (which as already noted does not appear to be a radiogenic form). For both the A-bomb and the ankylosing spondylitis cohorts, the Committee found that the data could be fitted approximately as well with either an absolute or relative risk projection model. The relative risk model was preferred since it was simpler and required less modifying factors. As noted earlier in this section, the use of a linear-quadratic model for leukemia gave a better fit to the LSS data, and incorporates an approximate DREF of about 2. However, since the dose at which the linear and quadratic components were equal (the "cross-over" dose) was estimated at about 90 rads, the DREF approaches unity as the doses drop below about 25 rads (i.e., as single-hits become predominant). Another significant departure from BEIR III Report was the Committee's conclusion that while the age at exposure is an important modifier of risk (i.e., the risk is greater from exposure of children), there was no longer any evidence to support earlier beliefs that those exposed before age 10 had a greater risk than those exposed between the ages of 10 and 20. The Committee's estimates of excess risk from 10 rems given to 100,000 persons (million person-cSv) as a function of age at exposure is summarized in Table 1.5.

Table 1.5. Relative Excess Risk of Leukemia Mortality per Million Person-cSv

<u>Age at Exposure</u>	<u>Excess Relative Risk</u>	
	<u>Males</u>	<u>Females</u>
5	111	75
15	109	72
25	36	29
35	62	46
45	108	73
55	166	117
65	191	146
75	165	127
85	110	73
Average for Population:	110	80

Table 4-3, BEIR V Report, (1990)

A final study that should be noted is the recent review of the leukemia risk among tinea capitis (ringworm of the scalp) patients by Ron *et al.*, (1988). Some 10,834 people, primarily Africans and Asians, were treated in Israel by X-irradiation of the scalp between 1948 and 1960 (average bone marrow dose of 30 rads). The average follow-up time is now about 26 years. Ron *et al.*, (1988) estimated that the relative risk of leukemia is now 2.8 (1.0-8.7, 95% CI) compared to controls, and 2.0 (0.6-7.6) compared to siblings. Comparable values for excess risk were 3.2 (0.1-13.7) among controls and 2.9 (-1.1- 18.9) among siblings. The absolute risk estimate was 0.81 (0.01-2.8) per million person-year-rads. As with other studies, the maximum period of risk was the five years following exposure.

1.5.1.1 *In Utero* Risk

There is one special group, however, worthy of discussion, since it may be one of the most radiosensitive groups for radiogenic leukemia: children exposed to radiation *in utero*. UNSCEAR (1988) reviewed the current data and concluded the risk from *in utero* exposure is still indeterminate, given that neither animal studies nor the Japanese data have confirmed a substantial increased risk of leukemia among offspring exposed *in utero*, regardless of trimester. In the interim, UNSCEAR continues to "prudently" assume a relative risk for leukemia of 1.58 based on its earlier 1986 report.

The NRPB, however, now feels that recent data (e.g., the Oxford Survey of Childhood Cancers, and the Japanese data) now permit a reasonable estimate of the relative risk of *in utero* exposure. Although the NRPB concluded that there are no substantial differences in sensitivity between trimesters, they also concluded that a relative risk of 1.4 was reasonable even though the 95% confidence interval on the data from Japan was large (1.14-13.5). The absolute risk for a low-LET radiation was estimated to be about 125 per million person-cGy for low-doses at low-dose-rate exposures *in utero*.

More recently, Yoshimoto *et al.*, (1988) reviewed 2,802 individuals exposed *in utero*, and using the DS86 dosimetry, concluded that the relative risk for the group was about 41% higher (i.e., a RR of 1.41), consistent with the NRPB estimate. However, it should be noted that there were only 13 leukemias found in this group, and the RR ranged from 1.24 (dose range of 1 to 29 rad) to 4.78 (over 60 rad). Yoshimoto also compared the RR for *in utero* exposure with children exposed between 0 to 9 years of age, and found no significant difference in risk, although the number of observed leukemias is small (2 cases), and subject to large uncertainty.

Brent *et al.*, (1987) reviewed the U.K. (Oxford) data and concluded the AR was on the order of 170 per million person-rem for the 10 years following exposure (during which essentially all of the risk is expressed).

A more recent review of the Oxford data by Gilman *et al.*, (1989), however, still concluded that there appears to be a greater risk from exposure during the first trimester (2.73 times higher than during the following two trimesters). However, since only 51 cases out of the 1,443 leukemias observed resulted from exposure during the first trimester, the statistical power is weak relative to the balance of the data (over 90 percent of which occurred following irradiation in the third trimester, which showed no statistically significant increase in

risk). Only time will tell whether the Oxford data will ultimately produce results that are consistent with results of other studied populations.

With regard to the risk of leukemia as the result of exposure *in utero*, the BEIR V Committee reviewed the available data and concluded that there is probably an association between *in utero* exposure and subsequent carcinogenic effects (largely leukemia) in adult life, but the magnitude of the risk remains uncertain. The Committee noted that two other studies indicated an approximate risk of about 200 to 250 deaths (half of them leukemia, and one fourth tumors of the nervous system) per million person-cSv in the first 10 years of life.

1.5.2 Breast

The most recent RERF study by Shimizu *et al.*, (1988) examined the cancer mortality data (1950-1985) based on the DS86 dosimetry. The relative risk from 100 rad was reported to be 2.19 (1.56-3.9, 90% CI); excess mortality (AR) was 1.2 (0.61-1.91, 90% CI) per million person-yr-rads.

In the UNSCEAR Report (1988), Table 69 gives the estimates of breast cancer risk based on the DS86 dosimetry. The lifetime risk for 1,000 women each receiving a dose of 100 Gy at high dose rate is 6.0 (2.8-10.5, 90% CI) per 100,000 person-cGy (relative risk), and 4.3 (2.2-6.9, 90% CI) per 100,000 person-cGy (absolute risk). Risk per person in a normal population would be about half the values reported for women. UNSCEAR concluded that the relative risk model with a linear dose response provided the best fit of the data. Canadian fluoroscopy patients also were found to exhibit a relative risk of about 2 - 3 for doses of 100 to 400 rads, with higher values for those exposed at a younger age. However, the Canadian data was found to be best fitted by a pure quadratic model, although a linear-quadratic fitted nearly as well. UNSCEAR also examined the data from women treated with radiation therapy for breast cancer, and found the data equivocal. For example, one study reported relative risks of 1.2 to 1.4 after five years of follow-up, while a second study of the same population reported a relative risk of 2.0 (1.9-2.1, 95% CI) for a second tumor vs. 1.5 (1.5-1.6, 95% CI) for women treated without radiation. However, for those women surviving 20 years or more after irradiation the RR was 1.7 but was no longer statistically significant. Subsequent studies of women receiving radiation therapy for breast cancer have shown little or no evidence of statistically meaningful excess risk. Much of the uncertainty has been attributed to the possible application of unreported chemotherapy which could have modified the radiation response.

In 1988, the National Radiological Protection Board (NRPB, 1988) estimated the risk of breast cancer from irradiation. Given the positive results reported by RERF, it was somewhat surprising to find their estimate based on studies of western women. They concluded that the excess lifetime relative risk of mortality for high dose-rates (>10 cSv/day) of low-Let radiation was on the order of 1.1 following exposures of 10,000 person-cGy. (about twice that estimated from the Japanese data). For dose rates below 10 cGy per day, the NRPB recommended a dose effectiveness reduction factor of 2 (i.e., an excess lifetime relative risk of 55 per million person-cGy). In the United States, mastitis patients exhibited a relative risk of about 3.2 (2.3-4.3 90% CI) following irradiation of a single breast, or about twice that for irradiation of both breasts (Shore *et al.*, 1986).

The BEIR V Committee reviewed the current data on risk of breast cancer (incidence and mortality) and concluded:

- 1) The development of cancer from irradiated mammary target cells is dependent on the hormonal status of the cells over time;
- 2) Breast cancers resulting from irradiation are similar in age distribution and pathology to those that appear spontaneously;
- 3) Women irradiated when less than 20 years of age have higher risks of breast cancer than those irradiated after 20 years of age;
- 4) Epidemiological data do not demonstrate a dose-rate effect;
- 5) The evidence is lacking of an increased risk of breast cancer for exposure before age 20, and little evidence of an increased risk after age 40;
- 6) Pregnancy and lactation before age 20 may reduce the risk of radiation-induced cancer;
- 7) The human data do not show a relationship between dose and latency and
- 8) Latency is at least 10 years regardless of age at exposure.

The BEIR V Committee preferred a linear, relative-risk model that varies with age at exposure and time after exposure. The risk of radiogenic breast cancer mortality among females as a function of age at exposure and time after a single 10 rem dose is summarized in Table A.3 of Appendix A to this report. The excess relative risk ranges from about 0.105 (45 years post-exposure) to about 0.427 (25 years post-exposure) for those exposed at ages 5 to 15. Comparable risks for those exposed at age 25 range from 0.014 to 0.057. Lifetime mortality risks from a dose of 10 cSv to 100,000 persons (million person-cSv) as a function of age at exposure are summarized in Table 1.6.

1.5.3 Lung

Shimizu et al., (1988) reported that the linear response and relative risk models provided the best fit of the data for Japanese A-bomb survivors. Estimates of relative and absolute risks at 100 rads were 1.63 (1.35-1.97, 90% CI), and 1.68 (0.97-2.49, 90% CI) per million person-yr-rads, respectively. These estimates employed the most recent DS86 dosimetry.

The UNSCEAR Report (1988) concluded that for brief low-LET exposures (primarily Japanese A-bomb survivors and ankylosing spondylitis patients), the relative risks of lung cancer mortality were about 1.2 to 2.0. Also noted was a recent study by Boivin and O'Brien (1988) of second cancers following irradiation of patients suffering from Hodgkin's disease, which showed that the relative risk of tumors of the respiratory system was about 2.5. The UNSCEAR Committee's estimates of lifetime lung cancer risk based on the DS86 dosimetry are presented in UNSCEAR Table 69 for 1,000 people exposed to 100 cGy each at a high dose rate; 15.1 (8.4-23, 90%CI) per 100,000 person-cGy (relative risk), and 5.9 (3.4-8.8, 90% CI) per 100,000 person-cGy (absolute risk).

The NRPB (1988) using the DS86 dosimetry, and assuming a neutron RBE of 10 (although the actual risk is relatively unchanged by the neutron dose

Table 1.6. Relative Excess Breast Cancer Mortality per Million Person-cSv

<u>Age at Exposure</u>	<u>Excess Relative Risk</u>
5	129
15	295
25	52
35	43
45	20
55	6
65-85	-
Average for Population:	70

Table 4-3, BEIR V Report, (1990)

contribution), estimated the excess lifetime relative risk of lung cancer, from high-dose-rate exposures of a low-LET radiation, to be 3.5 per 10,000 person-cGy for the population of the United Kingdom (i.e., about twice estimate in the UNSCEAR Report, 1988). The NRPB recommended a dose-rate effectiveness factor (DREF) of 3, bringing the total lifetime relative risk of lung cancer from low-dose-rate exposure to low-LET radiation to about 120 per million-person-cGy [or 6 times that used by the ICRP, (1977) in setting up the current risk-based radiation protection system].

The BEIR V Report (1990) reviewed the current data relating risk of respiratory cancers to radiation exposure from three studies: the A-bomb survivors (LSS cohort); the ankylosing spondylitis study (ASS cohort); and the uranium miners and other underground miners exposed to high LET radiation from short-lived radon progeny. The Committee concluded that the excess relative risk from a single 10 rem dose (regardless of age at exposure) ranged from about 0.02 to 0.20 as a function of sex and time after exposure. The risks are summarized in Table A.3 of the Appendix to this report. Lifetime excess relative mortality risks from a single dose of 10 cSv given 100,000 persons (million person-cSv) as a function of age at exposure are summarized in Table 1.7.

1.5.4 Thyroid

The latest RERF report by Shimizu et al., (1988) indicates that there has been no statistically significant increase of thyroid cancer among the Japanese bomb survivors.

The UNSCEAR Report (1988) concluded, however, that there are some data to indicate that children are at increased risk of thyroid cancer following irradiation. Quoting from studies of children irradiated for ringworm of the scalp (*Tinea capitis*), enlarged thymus, hemangiomas, enlarged tonsils, and children exposed to heavy fallout from weapons tests near the Marshall Islands, UNSCEAR indicates the risk ranges from less than 0.1 percent to about 8 percent from doses in the range of from 9 cGy to as high as 2,000 cGy at 30 years after exposure. The excess risk was estimated to be in the range of 1.0 to 3.6 per million person-yr-cGy. However, in the UNSCEAR Report, Table 20 also showed that the doses received by several thousand children in the United States from the fallout from the tests of atomic weapons were in the range of 30 to 240 cGy with no observable increase in risk at the time of the study. The Committee's evaluation of the data from Japan (Table 69) did not provide an estimate of thyroid cancer risk, which, if present, was included in the estimate of the "remainder."

The NRPB quoted the National Council on Radiation Protection Report No. 80 (1985) as the basis for an estimate of the lifetime absolute risk of thyroid cancers from high-dose-rate, low-LET exposure to be 0.075 per 10,000 cGy for the population of the United Kingdom, with a dose effectiveness reduction factor of three for low dose-rates, and with an assumed mortality rate 10 percent. This estimate, 2.5 per million person-cGy, is to be compared with the value of 5 per million person-cGy as indicated in the ICRP Report No. 26 (1977).

The BEIR V Report (1990) reviewed the current data relating to the risk of radiogenic thyroid cancer and concluded that the A-bomb survivor data could not be used because of suspected bias in diagnosis of thyroid cancer. That left the Committee with only risk data from the *Tinea Capitis* Study in Israel, and the Rochester Thymus Study. Only cases appearing more than 5 years post-exposure

Table 1.7. Relative Excess Lung Cancer Mortality per Million Person-cSv

Age at Exposure	<u>Excess Relative Risk</u>	
	Males	Females
5	17	48
15	54	70
25	124	125
35	243	208
45	353	277
55	393	273
65	272	172
75	90	72
85	17	15
Average for Population:	190	150

Table 4-3, BEIR V Report, (1990).

were analyzed, based on the Committee's estimate of a minimum 20 year latent period. Since these groups only included patients irradiated as children, no adult data was available, and adult risk estimates were extrapolated from the childhood exposures.

The BEIR V Committee found that a linear dose-response function fitted the data best and, in view of the complications, made lifetime-risk projections based only on Israeli-born children who were over the age of 5 at time of exposure. The model predicts a relative risk of 8.3 for all ages per 100 cGy. On the basis of unpublished data supplied the Committee by RERF, it was concluded that the risk of radiogenic cancer is, at most, one-half that in children. The Committee concluded that the following general observations apply:

- 1) Radiation-induced thyroid cancer arises exclusively from the follicular epithelium, and has about a 10% mortality rate;
- 2) There are major differences in the spontaneous rates of thyroid cancer in different populations related, in part, to differences in life-styles;
- 3) Females are about 3 times more susceptible than males to radiation-induced and spontaneous cancer;
- 4) The excess risk is greatest among children irradiated during their first five years of life and, for adults, the risk is at least half that for children;
- 5) Although the data are fitted best by an absolute risk model that includes allowance for cohort effects, latency, age at exposure, and sex, the Committee concluded a relative risk model is preferred because of the strong dependence of risk on the spontaneous incidence;
- 6) The risk ratio, for exposures to ^{131}I vs. X-rays, was estimated as 0.66, with a broad 95% confidence interval (0.14-3.15); and
- 7) Progression from an initiated cell to a thyroid neoplasm is strongly dependent on hormonal balance.

1.5.5 Bone

The recent analysis of radiation risk for the Japanese A-bomb survivors (Shimizu et al., 1988) indicated no statistically significant increase in bone cancer. Similar negative results for exposure to a low-LET radiation were reported in the UNSCEAR Report (1988) and by the NRPB (1988). Presumably such cancers, if they arose, would be in the "remainder" category.

The UNSCEAR Report (1988) also noted that about 27 bone sarcomas could be projected (AR) from the German ankylosing spondylitics treated with Ra-224 (doses ranged from 205 cGy in adults to 1,100 cGy in children). The Report also noted that a recent study of second cancers among Hodgkin's disease patients treated by radiation therapy alone indicated that a relative risk of 20 was observed for bones and joints (Boivin and O'Brien, 1988).

The NRPB lifetime absolute risk of bone cancer, which was estimated to be 0.05 per 10,000 person-cGy from a low-dose-rate exposure, was based upon neoplasms among radium dial painters, and therefore is of questionable value for

estimating risks from a low-LET radiation because of the large differences in cellular effects from α - vs. β -particle or photon irradiation.

Boice et al., (1988) reported a suggestion of an increased relative risk among women receiving low LET radiotherapy for cancer of the cervix (RR = 1.3).

The BEIR V Report (1990) reviewed the current data on radiogenic bone cancer, and declined to estimate the risks from low LET irradiation in human populations. The Committee reached the following general conclusions:

- 1) Although human data indicate bone cancer may be induced by large doses of X- or γ -rays, the dosimetry involved has been too uncertain to permit precise estimates of risk;
- 2) The LSS data provide no evidence of excess bone cancer resulting from γ -ray doses as high as 400 cGy;
- 3) The most definitive dose-response relationship for radiation-induced bone cancer comes from exposures to internally deposited α -emitters, ^{224}Ra and ^{226}Ra , for which the excess lifetime risk was found to be about 0.02 per 100 person-cGy; and
- 4) The studies with beagles indicate a non-linear, concave upward dose-response relationship from internally deposited ^{90}Sr , the risk from ^{226}Ra was estimated to be up to 25 times greater per unit dose than for ^{90}Sr .

1.5.6 Liver

Neither the data of Shimizu et al., (1988) nor those in the UNSCEAR Report (1988) indicate statistically significant increases in the risk of liver cancer among the Japanese A-bomb survivors or other populations (e.g., cancer therapy patients whose livers received substantial doses of a low-LET radiation. The occurrence of such cancers presumably would be included in the "remainder" category.

The NRPB presented an estimate of the risk of liver cancer based on the data in the BEIR IV Report (1988) of patients treated with thoratrast, ^{224}Ra , primarily in Germany. Using an absolute-risk model, the NRPB estimated that lifetime mortality risk from low-dose-rate doses to the liver would be about 15 per million person-cGy. As already noted for the bone, the value of such estimates for low-LET exposures is questionable.

Although most of the data related to the risk of liver cancer comes from people exposed to α -emitting, internally-deposited radionuclides (e.g., thorium), the BEIR V Committee also reviewed the current LSS and ASS cohort data. They found that the total liver cancer deaths among the A-bomb survivors indicated a relative risk of mortality of about 1.26 per 100 cGy. However, when metastases to the liver from other cancers were excluded, there was no statistically significant increase in risk. Similarly, the Committee noted that the ASS cohort did not show a statistically significant risk from an estimated average X-ray dose of 163 ± 126 cGy, and no evidence of increased risk was found following radiotherapy for uterine cancer. The Committee noted that the thorotrast patient data indicate a risk of about 300 per million person-cGy, and that the risk from beta emitters may be about one tenth of that.

1.5.7 Lower Large Intestine/Colon and Rectum

The most recent RERF report (Shimizu et al., 1988) reports a statistically significant increase in the risk of colon cancer based on the DS86 dosimetry (assuming a linear dose-response). The estimated RR from exposure at 100 cGy was 1.85 (1.39-2.45, 90% CI), and the estimated excess mortality (AR) per 10,000 person-yr-cGy was 0.81 (0.40-1.30, 90% CI).

The UNSCEAR Report (1988) concluded that there was evidence for a risk of colon cancer in a study of over 180,000 women treated with radiation for cervical cancer and followed thereafter for 10 or more. The estimated relative risk at 100 cGy was 1.00 (0.00-1.02, 90% CI) for the colon, and 1.02 (0.99-1.09, 90% CI) for the cecum. The estimated absolute risk per million person-yr-cGy for the colon (none was provided for the cecum) was 0.01 (0.03-0.18, 90% CI). The rectum (like the cecum) received larger doses than the colon as a whole and consequently the estimates of the risk were somewhat more accurate. The Report listed the relative risk for rectal cancer following 100 cGy to be 1.02 (1.00-1.09, 90% CI), and the excess cases per million person-yr-cGy to be 0.06 (0.00-0.16, 90% CI). Based on the recent Japanese data and the DS86 dosimetry, the excess lifetime mortality due to colon cancer from exposure of 1,000 people to 100 cGy at high dose rates was estimated to be 7.9 (3.6-13.4, 90% CI) per 100,000 person-cGy (relative risk), and 2.9 (1.4-4.6, 90% CI) per 100,000 person-cGy (absolute risk). [See the UNSCEAR Report (1988), Table 69].

With the data for Japanese A-bomb survivors and the modified dosimetry, the NRPB (1988) estimated the excess lifetime relative risk of colon cancer for the population of the United Kingdom to be 1.1 per 10,000 cGy for high dose rates. Applying a DREF of 3, the NRPB estimated that the excess lifetime relative risk at low dose rates would be about 37 per million person-cGy.

In the BEIR V Report (1990), the data were reviewed of the risk of cancer of the digestive tract. The Committee concluded, based upon the Japanese A-bomb survivor data, that there was a significant increase of risk in the colon and the rectum only for large doses of a low-LET radiation, but that the dose-responses were quite uncertain. Nevertheless, it was estimated that the relative mortality risk was about 1.85 per 100 cGy, or 0.81 excess (absolute) deaths per million person-yr-cGy following a latent period in excess of 15 years. Excess relative risk from a single dose of 10 cGy ranged from about 0.011 to 0.14, depending on time after exposure and sex, for all ages at the time of exposure. The overall excess relative risks of all digestive cancers from an acute dose of 10 cSv to 100,000 persons (million person-cSv) are summarized in Table 1.8.

1.5.8 Stomach

Using a linear-effects model and a relative-risk projection model, Shimizu et al. (1988) reported that the total lifetime risk of mortality from stomach cancer to be 1.27 (1.14-1.43, 90% CI) for exposures at 100 cGy. The excess lifetime mortality was estimated to be 2.42 (1.26-3.72, 90% CI) per million person-yr-cGy, absolute risk.

The UNSCEAR Committee, relying on recent data for women treated for cervical cancer with radiotherapy, provided a relative risk estimate of 1.69 (1.01-1.02, 90% CI) at 100 cGy, and an estimated excess mortality of 3.16 (0.05-10.4, 90% CI) per million person-yr-cGy. A recent study of Hodgkin's disease patients treated by radiotherapy alone indicated a relative risk of 1.8

Table 1.8. Relative Excess Mortality Risk from Cancers of the Digestive Tract per Million Person-cSv

<u>Age at Exposure</u>	<u>Excess Relative Risk</u>	
	<u>Males</u>	<u>Females</u>
5	361	655
15	369	653
25	389	679
35	28	73
45	22	71
55	15	64
65	11	52
75	5	26
85	-	4
Average for Population:	170	290

Table 4-3, BEIR V Report, (1990).

for cancers of the digestive system (Boivin and O'Brien, 1988). The UNSCEAR Committee's estimate of the excess lifetime mortality risks from stomach cancer based on the recent Japanese data (Table 69) are; 12.6 (6.6-19.9, 90% CI) per 100,000 person-cGy (relative risk) and 8.6 (4.5-13.1, 90% CI) per 100,000 person-cGy (absolute risk) delivered at high dose rate.

The NRPB, using the recent data from Japanese bomb survivors (relative risk, linear effects models) estimates the lifetime relative risk of stomach cancer mortality to be 0.73 per 10,000 cGy at high dose rates, or 24 cases per million person-cGy at low dose rates.

The BEIR V Committee reviewed the current data on risk of stomach cancer, and concluded the best evidence comes from the LSS cohort, although other studies also show increased risk. The LSS data indicate an excess relative risk of 0.23 per 100 cGy, and the absolute excess risk of 2.09 deaths per million person-yr-cGy is the largest excess risk observed for any site, next to leukemia. The BEIR V report noted that the University of Chicago study of patients irradiated for peptic ulcer indicates an absolute excess risk of 5.5 per million person-cGy, based on a life table analysis. The Committee concluded that its estimate of relative risk of mortality, 1.19 per Gy, is appropriate. The risk of stomach cancer was included in Table 1.8 in the preceding section under risk of "digestive cancer."

1.5.9 Other Organs

The BEIR V Committee considered the risk of radiogenic cancer to a number of other organs, some of which are discussed below. However, the Committee also evaluated the data for several other organs that were not explicitly considered by other studies. These include cancers of the brain and nervous system, the testes, prostate, parathyroid glands, nasal cavity and sinuses, skin, pharynx, hypopharynx and larynx, small intestine, and salivary glands. Of these organs and tissues, only the skin and salivary glands were quantified by the Committee.

The Committee reviewed the risk of skin carcinomas, and even though such cancers carry a low risk of mortality, found that the risk may be higher than previously thought due to underreporting. Infants irradiated for enlarged thymus glands (average dose of 330 cGy) indicate that the average relative risk is on the order of 1.5 per 100 cGy, with an absolute excess risk in the range of 0.32 to 0.64 cases per million person-yr-cGy. The tinea capitis patients in Israel have now been followed for about 30 years, and data indicate a latent period of about 20 years, with an apparent enhancement of risk from ultraviolet irradiation of exposed skin. The estimated lifetime incidence risk of skin cancer on exposed face and neck was estimated to be about 3,200 cases per million person-yr-cGy.**

The Committee also reviewed the risk of cancer of the salivary glands as the result of medical irradiation and among A-bomb survivors. Among the A-bomb survivors, although there has been no detectable increase in mortality due to salivary gland cancer, the incidence has shown a dose-dependent increase that

**Note: if this estimate is truly an integrated lifetime risk, it would appear that the correct units would be person-cGy, and about one-third that for hair-covered scalp.

is somewhat smaller than seen among patients receiving radiotherapy. For those patients receiving medical irradiation (^{131}I and X-rays treatments), the results are consistent and yield an average relative risk of 6.9 (± 5.5) per 100 cGy after the first five years post-irradiation, with an excess risk of 0.26 cancers per million person-yr-cGy following doses in the range of 40 to 100 cGy (X-rays). The BEIR V Committee concluded that the average excess relative risk from salivary gland cancer is about 550% per 100 rads, and the average absolute excess risk is about 0.26 cases per million person-yr-cGy.

1.5.9.1 Female Genitals

No increased risk of genital cancers was reported by RERF (Shimizu et al., 1988) except for the ovary. The relative risk of ovarian cancer among the Japanese bomb survivors was estimated to be 2.33 (1.37-3.86, 90% CI) for doses of 100 cGy and the excess mortality, absolute risk, was 0.71 (0.22-1.32, 90% CI) per million person-yr-cGy.

The UNSCEAR Committee estimated that the relative risk of second cancers in females genitals (ovary, vagina, and other genital tissues) per 100 cGy, given during radiotherapy treatment for cancer of the cervix, was 1.01 (1.00-1.02, 90% CI), and the excess absolute risk per million person-yr-cGy was 0.05 (0.01-0.17). The Committee also noted that chemotherapy, for primary cancers, can complicate risk estimates. For example, in female Hodgkin's disease patients, a 2- to 5-fold increase in carcinoma of the cervix and anogenital region was observed following chemotherapy only (presumably from papilloma virus infections in patients with suppressed immune systems). The UNSCEAR Committee's current estimate of the excess lifetime mortality risks from ovarian cancer (Table 69) as the result of 1,000 women each receiving a dose of 100 cGy at high dose rate are; 3.1 (0.9-6.8, 90% CI) per 100,000 person-cGy (relative risk) and 2.6 (0.8-4.8, 90% CI) per 100,000 person-cGy (absolute risk). Risks for a normal population of both sexes would be about half those values.

The NRPB Report (1988) provided no estimates of the lifetime relative risk of cancer to female genitals, apparently lumping such cancers under "remainder."

The BEIR V Committee reviewed the current data regarding risk of radiogenic cancer of the ovary or uterus, and concluded that although there was evidence such risks exist, there were inadequate data to quantify the risk to the uterus. The strongest evidence for risk of cancer of the ovary comes from the A-bomb survivors, where the RERF estimated the relative risk to be 2.33 per 100 cGy, based on the DS86 dosimetry.

1.5.9.2 Urinary Bladder

Shimizu et al., (1988) estimated the risk of urinary bladder cancer among Japanese A-bomb survivors using the DS86 dosimetry. The relative risk for a dose of 100 cGy was estimated to be 2.27 (1.53-3.37, 90% CI), and excess deaths per million person-yr-cGy to be 0.68 (0.31-1.12, 90% CI), absolute risk.

The UNSCEAR Committee provided an estimate of the relative risk of bladder cancer among women treated for cancer of the cervix using radiotherapy of 1.07 (1.02-1.17, 90% CI) for doses of 100 cGy, and an estimated excess of cases (AR) of 0.12 (0.01-0.30, 90% CI) per million person-yr-cGy. The UNSCEAR Committee also provided excess mortality risks for bladder cancer (Table 69) based on the

DS86 dosimetry; 3.9 (1.6-7.3, 90% CI) per 100,000 person-cGy (relative risk) and 2.3 (1.1-4.0, 90% CI) per 100,000 person-cGy (absolute risk).

The BEIR V Committee reviewed the current data on the risk of cancer of the urinary tract following irradiation, and concluded that cancer of the bladder is the greatest risk, with some lesser risk of cancer of the urinary organs and kidneys. The Committee cited the recent RERF data which indicate a relative risk of urinary tract cancer of 2.3 per 100 cGy, and an absolute risk of 0.7 deaths per million person-yr-cGy.

1.5.9.3 Esophagus

Shimizu et al., (1988) reported a statistically significant increase in the risk of cancer of the esophagus among Japanese A-bomb survivors. The RR following a dose of 100 cGy was estimated to be 1.58 (1.13-2.24, 90% CI), while the AR coefficient was estimated to be 0.45 (0.10-0.88, 90% CI) per million person-yr-cGy.

The UNSCEAR Committee reported a relative risk of esophageal cancer for patients treated for breast cancer of 1.7 with radiation therapy, but no quantitative estimates of dose or statistical significance were provided. In addition, the results for the combined data for heavily irradiated sites as the result of treatment of ankylosing spondylitis and the Japanese Life Span Study indicated a statistically significant relative risk of 1.82 with 0.45 excess cases per 10,000 person-years, however, dose estimates are not provided. The ankylosing spondylitis data indicated a statistically significant relative risk for all times beyond 5 years post treatment, with an overall risk of 2.20. Another study of the spondylitis provided dose estimates, with an average dose of about 420 rads (5-810, 90% CI). The current UNSCEAR (1988) Committee estimates of the excess lifetime mortality for 1,000 persons exposed to 100 cGy of low-LET radiation at a high dose rate are 3.4 (0.8-7.2, 90% CI) per 100,000 person-cGy (relative risk), and 1.6 (0.3-3.1, 90% CI) per 100,000 person-cGy (absolute risk), based on the Japanese bomb survivors.

The BEIR V Committee examined the current data on the risk of esophageal cancer from irradiation, but declined to make their own estimate of risk. The Committee cited the RERF estimated relative risk of 1.58 per 100 cGy, and found study results among the ASS cohort and women treated for cancer of the cervix consistent with the RERF findings. The risk of esophageal cancer is included in the Committee's overall estimated risk of cancers of the digestive tract (see Section 1.6.7 and Appendix for more details).

1.5.9.4 Multiple Myeloma

Shimizu et al., (1988) estimated that the relative risk to the A-bomb survivors at 100 cGy (organ dose) is 3.29 (1.67- 6.31, 90% CI), while the excess mortality (AR) is 0.26 (0.09-0.47, 90% CI) per million person-yr-cGy.

The UNSCEAR Committee noted that the risk of multiple myeloma among Japanese bomb survivors exhibits a 15 to 25 year latent period followed by an extended period of risk. Early estimates of risk based on the T65D dose estimates indicated the relative risk ranged from about 1.6 to 2.3 and excess absolute risk was about 0.48 per million person-yr-cGy. The UNSCEAR (1988) Committee now estimates the risks of mortality from multiple myeloma following exposure of 1,000 people to 100 cGy each delivered at high dose rate are; 2.2

(0.6-5.1, 90% CI) per 100,000 person-cGy (relative risk) and 0.9 (0.3-1.7, 90% CI) per 100,000 person-cGy (absolute risk), based on the survivor data.

The NRPB provided no risk estimate for multiple myeloma, but instead included these neoplasms with other cancers in the "remainder."

The BEIR V Committee reviewed current data on the risks of lymphoma and multiple melanoma, and concluded that the major risk is from multiple myeloma. The Committee noted that the A-bomb survivor data shows a relative risk of multiple myeloma of 3.29 per 100 cGy, with a corresponding absolute excess risk of 0.26 per million person-yr-cGy.

1.5.9.5 Pancreas

Shimizu et al., (1988) did not indicate any statically significant increased risk of cancer of the pancreas among the Japanese A-bomb survivors. The same is true for the current UNSCEAR Report (1988), and the NRPB Report (1988).

The only statistical indication that radiation may possibly cause cancer of the pancreas comes from cervical cancer patients treated by external X-ray therapy or implantation of intra-cavity radium sources with cervical doses in the range of 2,000 to 7,000 rem over a 4 to 8 week period [UNSCEAR Report, (1988), Table 26]. Risk estimates for women followed for 10 years or longer post-treatment indicate that the relative risk for doses on the order of 100 cGy is 1.00 (0.72-1.62, 90% CI), while the absolute risk is 0.00 (-0.65-1.43). Thus, the statistical indication is that the probable risk is indistinguishable from zero, but recognizing that many of the women treated are still being followed and that many person-years of experience still lie ahead, it appears possible some small level of risk may ultimately be detected.

The BEIR V Committee reviewed the current data and concluded that there are no study cohorts which have exhibited a reliable, quantified estimate of risk, suggesting that this organ is relatively resistant to radiation carcinogenesis.

1.5.9.6 Kidney

As was the case for the pancreas, none of the current major Reports--RERF, UNSCEAR or the NRPB--indicated a statistically meaningful risk of cancer of the kidney from exposure to low-LET radiation.

Only in the case of cervical cancer patients treated by radiation therapy (see Subsection 1.5.9.5 above) has there been a reasonable indication of an increased risk. The UNSCEAR Report (1988) presented the relative and absolute risk estimates (Table 26) as 1.71 (1.03-3.24, 90% CI) at 100 cGy, and 1.10 (0.06-3.50, 90% CI) per million person-yr-cGy, respectively. Based on these data, it appears likely that the kidney will present a statistically meaningful risk from irradiation, although it will be some time before adequate follow-up allows a reliable estimate of risk.

The BEIR V Committee provided no estimate of the risk of cancer of the kidney, but included it in the cancer category, "urinary tract" (see 1.4.9.3).

1.5.10 Summary of Current Estimates of Human Risk

The results of the major studies to date are summarized in Table 1.9. It should be noted that the totals by organ plus leukemia are not precisely the same as the totals from leukemia and nonleukemia. That is not surprising given the rounding and averaging of values for sexes and populations inherent in these estimates. Given the substantial differences in modeling approach and selection of projection models and DREFs, the results of these three recent studies are in reasonably good agreement. Although the most recent report of the United States Nuclear Regulatory Commission (USNRC Report, 1989) was not reviewed due to its recent publication, its central estimate is in reasonable agreement with these results, estimating that the risks per million person-cSv are about 48 for leukemia, absolute risk, and range from about 410 to 830 for nonleukemias, relative risk, for a total risk of about 460 (non-age specific) to 880 (age-specific) cancer deaths per million person-cSv.

1.6 RADIATION ONCOGENESIS IN SPECIFIC ORGAN SYSTEMS IN ANIMALS

Excellent animal models for radiation-induced neoplasms of some tissues exist and are capable of being manipulated both *in vivo* and *in vitro* to study oncogenesis at low doses and to study expression of already transformed cells and host factors. It is logical to choose models which parallel those of primary human concern, that is, those tumors which are most likely to be induced after low-dose exposure. Risk estimates based on neoplasms of the breast, bone marrow, lung, and thyroid could represent realistic and defensible ones for radiation protection purposes. With this idea in mind, this Section will concentrate on animal data which deal with the aforementioned systems, except where other systems (such as skin) may have the potential of offering unique contributions relative to our understanding of radiation oncogenesis.

This Section is not intended as an exhaustive review of what is known about each of the specific systems discussed, but is intended to bring out concepts that may be critical for understanding basic mechanisms of radiation oncogenesis and which may be important in the overall consideration of risk estimation. Only major reviews and selected papers are referred to in this regard.

1.6.1 Hematopoietic System

The hematopoietic tissues are sensitive to the oncogenic effects of ionizing radiation in both humans and a variety of animal species. Early recognition of the leukemogenic effects of radiation led to much research in this area as summarized below.

1.6.1.1 Model Systems

Two species have been used for the most critical studies of radiation leukemogenesis, the mouse and the dog. Many inbred and random bred mouse strains have been employed, and it is important to recognize that results have varied because of differences in genetic background of these animals. Beagles have been used almost exclusively for studies with dogs.

Table 1.9. Summary of Current Best Estimates of Major Risks of Cancer Mortality in Human Populations (Excess Deaths/10⁶ Person-cSv) from Low-LET Radiation

Type of Cancer	UNSCEAR (1988) ^a	NRPB (1989) ^b	BEIR V (1990)
Leukemia	93 (AR)	28 (AR)	95 (RR)
Nonleukemia	610 (RR)	420 (RR)	695 (RR)
Totals:	700	450	790
Breast	60 (RR)	55 (RR)	70 (RR)
Lung	151 (RR)	120 (RR)	170 (RR)
Thyroid	NA	2.5 (AR)	NA
Liver	NA	<1 (AR)	NA
Digestive Tract (Total)	239 (RR)	61 (RR)	230 (RR)
Esophagus	34 (RR)	NA	-
Stomach	126 (RR)	24 (RR)	-
Colon	79 (RR)	37 (RR)	-
Others	92 (RR)	163	260 (RR)
Ovarian	31 (RR)	NA	-
Bladder	39 (RR)	NA	-
Liver	NA	NA	-
Multiple Myeloma	22 (RR)	NA	-

^aBased on high-dose rate effects; no correction for low-dose rates.

^bAssumed 20 cSv/cGy for high-LET effects (bone, liver).

1.6.1.2 Viral Mechanisms of Leukemogenesis

The mouse has proved a fertile model for the study of viral oncogenesis, particularly for leukemogenesis. The finding that viral agents were associated with thymic lymphoma and that radiation could induce the same disease led to the concept that the radiation was simply a trigger for activation of the oncogenic virus (Yokoro, 1986). Partly from such work, the oncogene hypothesis emerged, and many studies over the past 20 years have addressed various aspects of murine leukemogenesis relating to viruses and other agents. Data are conflicting on the role of viral agents in murine leukemogenesis, however. The most recent thinking suggests that radiation and viral leukemogenesis may be independent processes and that a viral agent does not need to be involved in radiation-induced leukemogenesis (Newcomb et al., 1985; Yokoro, 1986).

1.6.1.3 Oncogenes and Chromosomal Changes

Radiation has been demonstrated to activate the K-ras oncogene in some thymic lymphomas in mice in contrast to chemicals which more usually activate the N-ras oncogene (Guerrero et al., 1984a). This activation was not found in nonmalignant tissues (Guerrero et al., 1984b). Later studies demonstrated both K-ras and N-ras activation in some γ -radiation-induced thymic lymphomas, but none in neutron-induced lymphomas (Diamond et al., 1987). Dogs with γ -radiation-induced myeloproliferative disorders were shown to have activated oncogenes in three out of four cases (Frazier et al., 1987).

Chromosomal aberrations associated with oncogene activation are now well established as important for hematopoietic oncogenesis (Nowell and Croce, 1988; see Task 2). As far as radiation-induction is concerned, causal associations between chromosomal changes and experimentally induced tumors have not yet been established. Partial deletions of chromosome 2 have been found in several studies of radiation-induced myeloid leukemia in mice (Fry and Storer, 1987). Some chromosome 2 rearrangements were thought to confer an altered cellular phenotype and possibly to represent an initiating event for murine, acute, myeloid leukemia (Silver et al., 1987; Silver et al., 1988). Recent studies indicate that the chromosome 2 rearrangement is in multipotential hematopoietic cells, and that the interleukin 1 (IL-1) α and β genes are located close to the critical chromosome 2 breakpoint (Silver et al., 1989). Other changes, such as chromosome 15 trisomy, have been reported for thymic lymphoma (Diamond et al., 1987).

1.6.1.4 Growth Factors

It is clear that growth factors like interleukin 1 (IL-1) play a role in the regulation of hematopoiesis (Le and Vilcek, 1987). IL-1 apparently controls differentiation of both normal and leukemic myeloid precursor cells (Lotem and Sachs, 1988; Lotem and Sachs, 1989). Silver et al. (1989) demonstrated that the IL-1 beta gene is specifically deregulated in radiation-induced, chromosome 2 rearranged, acute myeloid leukemias in mice, suggesting an important role for these growth factors.

1.6.1.5 Other Factors

As has been pointed out when discussing radiation leukemogenesis, more than one disease is involved (Yokoro, 1986). Thymic lymphoma, a disease involving T lymphocytes, is the most common type of hematopoietic neoplasm in the mouse and

is readily induced by radiation. Myeloid leukemia is less common and is a disease of the granulocytic cells of the bone marrow. There are large strain differences in the natural incidence and in the radiation-induced incidence of these diseases. Different patterns of irradiation can have vastly different effects on these tumors. For example, shielding a portion of the bone marrow reduces thymic lymphomagenesis, but not myeloid leukemogenesis. Thymectomy before or shortly after irradiation eliminates thymic lymphoma but has no effect on myeloid leukemia, whereas splenectomy reduces myeloid leukemia but has little effect on thymic lymphoma.

Influences outside the hematopoietic system proper also affect radiation leukemogenesis. Ovariectomy in RFM strain of mice has been demonstrated to reduce dramatically the incidence of thymic lymphoma. This result was related to hormonal imbalances demonstrating the hormonal dependence of this neoplasm and the importance of host factors in the expression of the final incidence of neoplasia (Storer et al., 1982).

Aspects of the various stages of the radiation oncogenic process have been addressed. Mole (1984), in studies of myeloid leukemogenesis, adopted the proposal of Gray (1965) that cellular inactivation is a critical factor and must be accounted for in the quantitation of tumor induction. These studies, and those of Upton et al. (1970), demonstrated that the initial transforming event after irradiation is not permanent but may "decay" with the passage of time unless "fixed" in some fashion (Mole, 1986). The cellular studies of Han et al. (1980) suggest that the "decay" of tumor induction with dose protraction was due to intracellular repair.

Studies of chronic irradiation and leukemogenesis have stressed the possible role of radiation in stages beyond initiation. Both myeloid and lymphoid neoplasia can be induced in chronically irradiated mice; a heightened proliferative activity and an overcompensatory regeneration of both myeloid and lymphoid precursors was felt to be involved as a promotional effect (Cain and Rosenblatt, 1985). In chronic radiation myeloid leukemogenesis in the dog, initial hematopoietic suppression was followed by recovery, in part mediated by the acquisition of "radioresistance" of vital hematopoietic precursors (Seed et al., 1985). Amplification of such resistant clonal cells was a critical factor in leukemogenesis. If radiation exposure was terminated rather than continued for the lifespan of the dogs, the incidence of leukemia was greatly reduced. There were also sequential changes in various clonal types of cells during the process. All of these findings are consistent with a multistaged process in leukemogenesis with the protraction of the irradiation playing a role in the promotion and progression of the neoplastic process.

1.6.2 Mammary Gland

Spontaneous breast cancer is a neoplasm of major importance in women and the breast has been shown to be sensitive to radiation-induced neoplasia in people and in animals. Experimental studies have shown the involvement of a number of factors, especially hormones, which play a role in oncogenesis in this organ.

1.6.2.1 Model Species

Mammary oncogenesis is dependent on a number of factors in the mouse, including viral, hormonal, genetic, immunologic, and environmental factors

(Shellabarger et al., 1986). Mammary oncogenesis in the rat is also affected by a variety of factors, but viruses do not appear to play a significant role; endocrine factors are of paramount importance. Most of the critical work on mammary oncogenesis has been with these two species.

1.6.2.2 Viral Mechanisms of Mammary Oncogenesis

It has been suggested that virtually all mice have the mouse mammary tumor retrovirus (MTV) present in their mammary tissues in some form and that mammary oncogenesis, whether induced by physical or chemical agents, depends on the presence of this virus (Shellabarger et al., 1986). Radiation induction of mammary tumors is strain-dependent, and strains that have no free virus have a low spontaneous frequency and a low radiation-related susceptibility to the induction of mammary neoplasia. Studies that have suggested any abscopal mechanism for radiation oncogenesis in the mouse should be evaluated in this context.

1.6.2.3 Hormonal Influences

The effect of hormones on mammary oncogenesis in the rat has been studied extensively (Shellabarger et al., 1986). A variety of manipulations that affect hormonal balance such as: ovariectomy (Solleveld et al., 1986); estrogen treatment (Stone et al., 1980; Janssens et al., 1981; Ito et al., 1984; Shellabarger et al., 1983; Solleveld et al., 1986); and the implantation of prolactin-secreting pituitary tumors (Yokoro et al., 1980) can change the incidence of radiation-induced mammary neoplasms.

Ovariectomy has been shown to reduce the oncogenic response to radiation. The response to estrogen treatment varies with the strain of rat and the type of mammary tumor induced (Shellabarger et al., 1986), but generally estrogenic substances increase radiation-induced mammary tumorigenesis in a synergistic fashion. It has long been known that prolactin acts synergistically with radiation in the induction of rat mammary tumors. In fact, the evidence indicates that the effects of estrogens are actually mediated through their ability to increase prolactin in treated animals (Shellabarger et al., 1983). Prolactin-secreting pituitary tumors grafted into irradiated rats increased tumor yields even when the grafts were performed many months after the radiation exposures (Yokoro et al., 1980). A similar phenomenon was noted for treatment with diethylstilbestrol (Shellabarger et al., 1983). These studies indicate that the hormonal effects are probably promotional in nature and that cells initiated by the radiation can persist for a considerable period of time until a promotional factor is applied. Tumors induced by both low- and high-LET radiations demonstrate these interactions with hormones (Shellabarger et al., 1986).

1.6.2.4 *In Vitro/In Vivo* Studies

Of very real importance to the study of radiation oncogenesis is the recent development of so-called *in vitro/in vivo* experimental techniques. These have been particularly useful in addressing some basic questions. The systems that have been used most have involved the mammary gland, tissues of the respiratory tract, and the thyroid gland.

Studies with the mammary gland have used enzymatically dissociated mammary cells from carcinogen-treated mice (Ethier et al., 1984; Ethier and Ullrich,

1984; Ullrich, 1986; Adams et al., 1987) and rats (Clifton et al., 1986) to evaluate initiation and progression of radiation-induced mammary tumors. When injected into the subcutaneous fat pads of isologous hosts, the dissociated cells form mammary outgrowths assumed to be derived from single cells, referred to as "clonogenic" cells (Clifton et al., 1986). When taken from animals previously treated with a carcinogen, such as radiation, these outgrowths can display dysplastic changes similar to those seen during mammary oncogenesis *in vivo* (Ethier et al., 1984a). Dysplastic lesions were detected after low doses of radiation, and the cells that had altered phenotypes could be detected within 24 hours after irradiation. A progressive change to greater autonomy of growth occurred with time; that is, the longer the period between radiation exposure and cell dissociation/transplantation, the greater the incidence of lesions and the longer that their dysplastic growth patterns were maintained (Ethier and Ullrich, 1984b).

Further studies revealed that, with increased time *in situ* between exposure and dissociation, an increased tumorigenic potential was developed when such cells were first incubated *in vitro* and then retransplanted back into mice (Adams et al., 1987). These studies suggest that altered growth potential was acquired early after irradiation, while the ability to maintain autonomous growth was a separate event. Further, although altered cells could be found in 100% of irradiated donor mice, only 14% of mice given the same radiation dose developed mammary neoplasms (Adams et al., 1987). Thus, it appeared that initiation was more common than tumor induction.

Clifton et al. (1986) used a protocol in which host rats were hormonally manipulated to maximize mammary oncogenesis (i.e., hyperprolactinemic). Known numbers of dissociated mammary gland cells from previously irradiated or control rats were transplanted into the fat pads of the hosts. Some of the rats that received the control mammary cells were irradiated 35 days later. The incidence of mammary carcinomas at the site of the grafts were evaluated. The results indicated that radiation initiation was a common cellular event, but that both incidence of neoplasms and the latent period were functions of the number of target cells, mammary clonogens, available at the time of the initiating irradiation.

Finally, Ullrich (1986), using the method of transplanting dissociated cells into mice, found that, after low dose-rate exposures to fission-spectrum neutrons (1 cGy/day), there was a greater degree of persistence of mammary dysplasias than after high dose rates (1 cGy/min.). This was true even though the overall incidence of dysplastic lesions was similar between the two treatments. This observation suggested that low-dose-rate neutrons enhanced the probability of the promotion/progression of radiation-altered cells rather than increasing the numbers of initiated cells.

1.6.3 Respiratory System

Lung cancer is another major human neoplasm and it is known to be induced by ionizing radiation. Animal models of respiratory system neoplasia have revealed important data relating to dose localization and some of the cellular mechanisms of induction by radiation.

1.6.3.1 Model Species

Studies of radiation-induced lung cancer have employed a wide variety of animal models, but the most important of these have included the rat, the mouse, the Syrian hamster, and the dog. This is partly the case because there is no single model that exactly mimics the pattern of human lung tumor development, and partly because there is a considerable difference between the patterns of lung tumor development that occurs between animal species (Bair, 1986).

1.6.3.2 Localization of Dose

Much more so than the situation with hematopoietic tissues, or tissues of the mammary or the thyroid glands, the spatial pattern of dose delivery to the lung is a significant factor in oncogenesis (Bair, 1986). This is partly because studies using external exposures have not appeared to be as effective in inducing lung tumors as those using internal emitters. External exposures are usually assumed to deliver a relatively uniform dose to the lung, whereas, with radionuclides deposited in the lung, uniform exposure is rare. The least uniform irradiation is that delivered by α -emitters because the particles have a relatively short range in tissue. Patterns of deposition, clearance, and retention of radionuclides play a critical role in determining dose, and these are dependent on the species of the animal used (Griffith *et al.*, 1986). When coupled with the fact that each species has a significantly different spontaneous incidence of lung tumors, which also differ in histological type, broad generalizations are difficult to arrive at.

One of the major questions relating to radiation-induced lung cancer in the past 15 years has been the so-called "hot particle" problem (BEIR, 1976). One assumption was that the localization of dose from high-specific-activity particles of α -emitting radionuclides, such as plutonium, would increase the risk substantially compared with a similar dose spread more uniformly throughout the respiratory tract. Virtually all of the evidence that has been accumulated over the past decade has refuted the "hot particle" hypothesis and, in fact, seems clearly to show that uniform distribution of radiation dose is as effective or even more effective in causing lung cancer in a number of instances (BEIR IV, 1988). This result is assumed to be due to the greater degree of cell killing around "hot particles," reducing the number of viable cells available to express their initiation as compared with the situation when a more uniform distribution of the same dose is applied.

1.6.3.3 Dose Responses

As with other organ systems, different studies of external exposures to low-LET radiation have yielded dose-response curves that were linear or curvilinear in nature (Ullrich, 1980; Fry, 1981). In mice, lowering the dose rate can have an effect by reducing the effectiveness of tumor induction (Ullrich, 1980) and by apparently changing the response from a linear-quadratic to a linear one (Ullrich *et al.*, 1987). Neutron exposures in mice yielded curves that were linear at very low doses but could bend downward at doses as low as 20 cGy (Ullrich, 1983).

For internally deposited radionuclides, there is considerably more information on a wider range of species, but many of the critical studies in the dog are still in progress (Thompson and Mehaffey, 1987). Clearly, high-LET α -emitters are more effective than low-LET β -emitters in inducing lung cancer, as

would be expected (Bair, 1986). Interestingly, the curves for some studies with high-LET radiation have yielded curvilinear dose responses, rather than linear (Bair, 1986; Sanders et al., 1988). Sanders attributes some of this to temporal and spatial factors relating to the redistribution of particles of long-lived radionuclides in the lung of rats and suggests that the concurrence of inflammation, fibrosis, and metaplasia with carcinoma formation result in local factors which promote tumor formation to a greater degree at higher doses.

Studies with Syrian hamsters have shown that α -emitting radionuclide oncogenesis can be promoted by simply increasing cell division in the target cells by installation of saline solution over a period of weeks following exposure to the radioactive particles (Little, 1981).

Differences in the spatial and the temporal distributions of dose from internal emitters, as well as differences in radiation quality, can lead to the formation of different types of lung neoplasms. In dogs, α -emitters induce mostly bronchioloalveolar carcinomas (Bair, 1986), whereas β -emitters lead to a broader spectrum of tumors (Benjamin et al., 1975). In the latter case, dose rate plays a critical role in pulmonary oncogenesis (Hahn et al., 1983). Short-lived β -emitters, which delivered their dose at a high dose rate, tended to cause mostly carcinomas. As the dose rate decreased with longer-lived radionuclides, there was progressively a greater incidence of sarcomas, including hemangiosarcomas.

1.6.3.4 *In Vitro/In Vivo* Studies

Studies of carcinogen-induced transformation in the tracheal epithelial cells of the rat have revealed some interesting phenomena with respect to the effects of normal cell populations on initiated cells. Tracheal cells harvested from carcinogen-treated animals showed the characteristics of preneoplastic cells--that is, an increased growth capacity--and, after further changes, the capacity to produce neoplasms in appropriate hosts (Terzaghi et al., 1982a,b). It was demonstrated that normal cells can inhibit the growth of carcinogen-altered cells and/or affect the progression of these cells. This effect was probably mediated either through the production of a substance produced by normal cells, believed to be a transforming growth factor beta (Terzaghi-Howe and McKeown, 1986), or via cell-to-cell contact (Terzaghi-Howe, 1987). This situation appeared to be true for chemically-initiated cells (Terzaghi-Howe and McKeown, 1986; Terzaghi-Howe, 1987) as well as for radiation-initiated cells (Terzaghi-Howe, 1989). The degree to which this effect was observed was somewhat dependent on the LET of the radiation used, with apparently more inhibition being exerted on low-LET altered cells than high-LET altered cells (Terzaghi-Howe, 1989). Development of cellular resistance to transforming growth factor beta-induced differentiation is suggested to be an early, crucial step in radiation-induced transformation.

1.6.4 Thyroid Gland

The thyroid gland has been shown to be highly sensitive to radiation-induced neoplasia in humans and animals. Like the mammary gland, much has been learned about hormonal factors that affect radiation oncogenesis in the thyroid from animal studies.

1.6.4.1 Model Species

Studies of thyroid oncogenesis have been carried out primarily in the rat, although a few studies have utilized the mouse and the dog.

1.6.4.2 Radiation Quality

Based on both human experience and some animal data, current recommendations consider ^{131}I less carcinogenic in the thyroid gland, per unit of absorbed dose, than external radiation (NCRP, 1985). This is thought to be due to differences in dose rate and dose distribution within the gland. However, one study in the rat by Lee et al., (1982) did not demonstrate such a difference and showed a similar incidence in the frequency of carcinoma with ^{131}I and X-rays, with no differences due to dose rate from the two types of exposure. This question needs to be resolved.

1.6.4.3 Hormonal Influences

Thyroid gland function is under the control of the hypothalamic-hypophyseal system. A total feedback loop exists with the hypothalamus secreting thyrotropin-stimulating hormone which acts to stimulate the secretion of thyroid-stimulating hormone (TSH) which, in turn, causes the follicular cells of the thyroid gland to produce thyroid hormone, thyrotropin T3 and T4 (Clifton, 1986). TSH is the primary hormonal mitogen of thyroid cells (Clifton et al., 1986). Because of the lack of negative feedback, hypothyroid individuals, who produce abnormally low amounts of thyroid hormone, have an increased secretion of TSH. In turn, this stimulates proliferation of thyroid follicular cells. It is important to recognize that elevated levels of TSH alone can lead to increased risk for thyroid neoplasia in rodents as well as to an increased frequency of radiogenic tumors.

1.6.4.4 Age

The effect of age at the time of exposure on thyroid oncogenesis has been documented in people and supported in animal experiments. Children are more susceptible to subsequent neoplasia than individuals exposed as adults (NCRP, 1985), and rats (Christov, 1978) and dogs (Benjamin et al., 1987) showed increased risks when exposed as juveniles.

1.6.4.5 In Vitro/In Vivo Studies

A series of studies using both *in vitro* and *in vivo* techniques have been used to study mechanisms of radiation oncogenesis in the thyroid gland (Clifton, 1986). Monodispersed rat thyroid cells were shown to give rise to normal thyroid follicles when injected into the fat pads of host rats, some of which were thyroidectomized and hypothyroid. Follicles were derived from single thyroid clonogens and the proportion of grafted clonogens triggered to form follicles was dependent on TSH levels (Watanabe et al., 1983a).

In radiation studies, varying numbers of irradiated and unirradiated thyroid clonogens were injected, and the hormonal status of the hosts were manipulated. Neoplasms, particularly carcinomas, were observed to appear at the graft sites. These studies illustrated a number of important points. Quantitation of neoplasms arising from a known number of surviving, irradiated clonogens allowed quantitation of numbers of initiated cells, and gave direct

experimental evidence that initiation by radiation *in vivo* is a relatively common, rather than rare, cellular event (Mulcahy et al., 1984). The cell number used in the grafts influenced the frequency of neoplasia (Mulcahy et al., 1984). Expression was inversely related to the number of cells that were grafted in that, as the cell number went up, carcinoma frequency per grafted clonogen went down (Watanabe et al., 1988). It was also shown that the addition of unirradiated thyroid clonogens to the irradiated cell inocula further suppressed the expression of carcinomas.

Studies of hormone levels revealed that, as the inoculum number went up and/or unirradiated cells were added, newly formed thyroid follicles produced enough thyroid hormone to result in reductions of TSH (Watanabe et al., 1988). Thus, the initial hormone imbalance was playing a promoting role in thyroid oncogenesis. Further, when unirradiated thyroid clonogens were added to the inoculum, the probability of vascular invasion in the carcinomas (a measure of malignancy) was reduced. This observation suggests that hormonal factors can affect both promotion and progression of radiation-induced neoplasia.

Last, it is interesting that the clonogenic efficiency of donor cells from one-year-old rats was less than those from younger animals (Watanabe et al., 1983b), suggesting that age-related sensitivity was an inherent function of the donor cells themselves rather than other host factors.

1.6.5 Skin

While skin does not represent a tissue of either high risk for mortality or high sensitivity for human radiation oncogenesis, skin carcinomas are a risk at high doses. More importantly, animal models of skin oncogenesis have revealed some important and unique insights into mechanisms of radiation oncogenesis. Thus, a discussion of this organ system is appropriate here.

1.6.5.1 Model Species

Both the mouse and the rat have been used for studies of radiation oncogenesis in skin, although the rat has been shown to have a much greater radiosensitivity and to respond with a broader spectrum of neoplasms (Burns and Albert, 1986).

1.6.5.2 Promotion and Progression

Studies of multistage oncogenesis in mouse skin have helped to reveal the role(s) that ionizing radiation can play in this process. Using X-rays as an initiator, and a phorbol ester as a promoter, radiation did not increase the number of benign papillomas over that seen with phorbol ester treatment alone; however, radiation did increase the number of squamous cell carcinomas seen after long-term promotion. The occurrence of basal-cell carcinomas was dependent on the radiation dose but not dependent on promotion (Jaffe and Bowden, 1986; Jaffe and Bowden, 1987).

In further studies, mice were initiated with a chemical carcinogen, promoted with phorbol ester, and then treated further with the phorbol ester or irradiated. Radiation did not increase the number of papillomas but did enhance the progression of preexisting papillomas to squamous-cell carcinomas (Jaffe et al., 1987). The results of these studies indicated that radiation can act as an initiator of skin oncogenesis but that there was no evidence that it had a

promoting function with respect to the production of premalignant papillomas. On the other hand, the ability of radiation to enhance the production of malignant squamous cell carcinomas from papillomas indicated that it can play a role in affecting the progression of the neoplastic process. Differences in activation of oncogenes has been reported between radiation and chemically-induced mouse skin tumors (Bowden et al., 1989).

These mouse studies are also important in that tumor-associated genes have been shown to be overexpressed in benign papillomas and squamous-cell carcinomas (Krieg et al., 1988). One of these oncogenes, transin, encodes for a secreted protease found in the carcinomas but not in premalignant papillomas. It was suggested that transin may play a role in the progression from the benign to the invasive, malignant state (Matrisian et al., 1986). Activation of other oncogenes has been reported in tumors of rat skin after irradiation (Sawey et al., 1987).

1.6.5.3 Dose Localization

The rat and mouse skin models have been used to address the question of the effectiveness of localized vs. uniform dose in radiation oncogenesis (Burns and Albert, 1986; Charles et al., 1988). As in the lung, the evidence suggests that uniform irradiation is more effective in inducing skin tumors. Also of interest is the finding that localized "hot spot" irradiation delayed the onset of tumors, but when the unirradiated tissue between heavily irradiated foci was exposed to a low dose of irradiation, the delay was eliminated (Burns and Albert, 1986). This observation was interpreted to mean that the proximity of unirradiated cells somehow affected the rate of progression of the irradiated cells to form neoplasms.

1.6.5.4 Interaction of Ionizing with Ultraviolet Radiation

Ultraviolet (UV) light is a known cause of skin cancer and there is evidence that UV and ionizing radiation interact to increase the risk of skin tumors in humans (Burns and Albert, 1986). Studies in rats have demonstrated that UV plus ionizing radiation resulted in more malignant tumors at lower doses of ionizing radiation (Burns and Albert, 1986).

1.7 RADIATION MUTAGENESIS AND GENETIC EFFECTS

Radiation is known to produce a variety of heritable changes in germ-line and somatic cells. This section addresses radiation induction of such changes in animals and people.

1.7.1 Experimental Mutagenesis in Animals

In a number of studies, mice and other vertebrates have been used to study radiation-induced heritable effects as reviewed below.

1.7.1.1 Studies with *Drosophila*

The end of World War II was accompanied by a greatly increased awareness of the biological effects of ionizing radiation exposure. The long-term genetic effects of radiation on the human population, even for the peaceful uses of atomic energy, was of principal concern. At that time, it was well known that radiation produces mutations (e.g., Muller, 1927), but virtually no information

was available for mutation induction in mammals in general, let alone in people. The early work using the fruit fly (Muller, 1927) indicated, for example, that mutations induced by X-rays were produced at a rate of about 1.5×10^{-6} mutations per locus per R, and that there was little effect of dose protraction on the yield of mutations per unit dose. The lack of a protraction effect, in retrospect, was not too surprising because many of the early experiments were carried out using mature and maturing male germ cells which are metabolically very inactive. One important conclusion from the early studies is that the vast majority of mutations are harmful. This conclusion has not changed over the years.

1.7.1.2 Specific Locus Mutation in Mice

The so-called "megamouse" experiments at the Oak Ridge National Laboratory and the U. K. Atomic Energy Establishment at Harwell were set up in the late 1940's and early 1950's to study radiation genetics in mammals. In both cases, the specific locus method was utilized where irradiated individuals from homozygous dominant stocks were mated to unirradiated individuals from homozygous recessive stocks. Seven specific loci controlling readily visible phenotypes were used by the Oak Ridge group, and six by the Harwell group. The data from these studies constitute the major part of our current knowledge of quantitative mammalian radiation genetics. The results have been reviewed by Searle (1974), and may be summarized as follows.

- (1) The low LET dose-response relationship for specific locus mutations due to high-dose-rate exposures of spermatogonial stem cells follows a general form that is often seen for the production of chromosomal exchanges in meiosis and for oncogenesis; i.e., there is an initial increase up to doses of about 600 cGy where a "saturation" occurs followed by a decreased yield at higher doses, 10 cGy. Very little data are available for low doses, but an adequate fit to the data that are available, as proposed by Abrahamson and Wolff (1976), would be,

$$I = 8.3 \times 10^{-6} + 0.69 \times 10^{-7} D + 0.66 \times 10^{-9} D^2$$

where I is the mutation frequency and D is the dose in cGy.

- (2) For protracted γ -ray exposures of spermatogonia, the mutation frequencies per unit dose (at ~300 cGy and ~600 cGy), decreased by a factor of about 2.5 to 3 when the dose rate was reduced from about 90 cGy per minute to about 0.8 cGy per minute. Further reductions to 0.009 or 0.001 cGy per minute did not decrease further the frequency per unit dose.
- (3) For females, oocyte mutation frequencies appeared to be fitted reasonably well by the expression (Lyon, et al., 1979),

$$I = 2.1 \times 10^{-6} + 6.0 \times 10^{-8} D + 0.9 \times 10^{-9} D^2$$

- (4) The dose response for low dose-rate exposures appears to be linear, i.e., a quadratic component is negligible.
- (5) Virtually no radiation-induced mutations were detected for oocytes ovulated seven or more weeks after irradiation.

- (6) Fission neutrons were about 6 times more effective in inducing mutations in spermatogonia, and about 5 times more effective in oocytes, for high dose-rate exposures. For low dose-rate irradiation, the RBE of neutrons relative to γ -radiation was about 20.
- (7) There was about a 35-fold variation in induced mutation frequencies among the different specific loci tested.

1.7.1.3 Other Mutations in Mice

Studies are also available on induced "dominant-visible" mutations and most of these appear to be lethal in the homozygous state. The radiation-induced frequencies are much lower than those observed for specific locus mutations.

Although valuable information on mammalian radiation genetics was derived from the "megamouse" experiments, it is quite unlikely that such an approach could be undertaken for quantitative estimates of mutation frequencies in the low-dose region, i.e., <10 cGy.

1.7.1.4 The Frequency of Mutant Alleles in the Population

A major concern about radiation-induced mutations in germ cells relates to the detrimental effects for future generations of increases in low-level radiation exposure of the human population. It is not simply a question of the increased risk for an expressed mutation in the first generation following exposure of an individual. The issue requires an estimate of the increased incidence if the whole, or a large, population, as well as future generations, receives an increase in radiation exposure.

It is well known that most mutations are harmful. Were it not for this fact, the frequency of individuals with a mutant phenotype in the population would continue to increase, even from exposure to background radiation. Because mutations are mostly harmful, they tend to reduce the "reproductive fitness" of individuals in which they are expressed, and such individuals suffer a selective disadvantage. The severity of the disadvantage depends on the mutant allele in question, but, in any case, this selective disadvantage will reduce the frequency of individuals bearing the allele in the next and future generations. If only the parently generation receives an additional radiation exposure (above natural background), then, at one extreme, it is possible to have dominant mutant alleles with high penetrance that are so deleterious that individuals bearing them are either sterile, or die before they reach reproductive age and are eliminated from the population during the first post-irradiation generation. Of course, this outcome would be modified for dominant alleles with lower penetrance, and/or a heritable change that has a less severe deleterious effect on reproductive fitness. Induced recessive alleles would raise the frequency of mutant phenotypes only slightly in the first generation because they would have to be homozygous for expression. Thereafter, the frequency of such individuals would decrease very slowly over hundreds of years because the frequency of mutant phenotypes, and their elimination by reduced reproductive fitness, would depend on the chance pairing of two relatively rare mutant recessive alleles.

The situation for an increase radiation exposure over the current and all future generations is that an equilibrium level is reached which is higher than that frequency observed in the first post-irradiation generation. Along with estimates of induced mutation rates from the mouse studies, a number of

assumptions have been made regarding numerical values for the frequency of expression of mutant alleles and the reproductive fitness, on average, of individuals bearing them to arrive at estimates of equilibrium frequencies. An example of the result taken from the BEIR III Report (1980) is shown in Table 1.10. This serves to illustrate that equilibrium levels can be considerably higher than estimates of mutation frequencies measured in the first generation.

1.7.2 In Vivo Detection of Mutations in Human Beings

New technologies have allowed researchers to address the difficult problems of measuring genetic changes in the cells of people. Significant insights in the future are possible from the application of some of the methods discussed in this section.

1.7.2.1 Somatic Mutations

Methodologies have been developed that permit measurement of spontaneous and induced mutations in human somatic cells *in vivo* (see Technologies for Detecting Heritable Mutations in Human Beings, 1986; Banbury Report 1987; Tate et al. 1989). Assays at four genetic loci have been described, all in either red blood cells or lymphocytes: (a) loss of activity of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) in lymphocytes; (b) loss in lymphocytes of certain antigens, designated HLA-A; (c) identification of new, specific, mutant hemoglobins; and (d) loss of alleles for a transmembrane protein of red blood cells, glycophorin A.

The *hgp*rt assay uses either an autoradiographic or a clonal method to measure mutation in lymphocytes (Albertini, 1982; Albertini et al., 1988). In the radioautographic method, mononuclear lymphocytes isolated from drawn blood are incubated with a mitogen to cause the initiation of growth and the synthesis of DNA. Incubation with 6-thioguanine (6-TG) kills cells that have a functional *hgp*rt gene--because 6-TG is a toxic analog of guanine, one of the bases in DNA--but allows the mutant cells to live. Radioactive thymidine, added to the mitogen-stimulated cells, is incorporated into the cells that remain alive and, consequently, the latter cells can be detected by standard radioautographic procedures.

In the clonal assay, mutation is scored by the fraction of lymphocytes that form colonies in medium containing 6-TG. As before, white blood cells are treated with PHA to induce growth. The cells are then inoculated into microtiter plates in medium with or without 6-TG. The number of mutants is scored after about 13 days incubation by scintillation counting of tritiated thymidine incorporation into DNA or by cell transfer and clonal expansion. The mutant frequency is taken as the ratio of the cloning efficiency in the presence of 6-TG to that in its absence. Background frequencies in the clonal assay of cells from different individuals vary substantially with values from 1.3 to 50×10^{-6} being reported, which introduces considerable uncertainty into the determination of induced mutation, and which can obscure induced mutations. An advantage of the clonal assay over the radiographic method is that mutant colonies can be analyzed further by isolating their DNA and determining the molecular nature of their mutations.

The validity of the *hgp*rt assay *in vivo* is subject to several caveats that pertain to analysis of mutation at this locus. Among the most important of these is the failure to detect large, extragenic, multilocus mutations, a kind of

TABLE 1.10. Genetic Effects of an Average Population Exposure of 1 cSv per 30-Yr Generation

Type of Genetic Disorder	Liveborn Offspring	Effect/Million Offspring	
		First Generation	Equilibrium
Single gene Autosomal dominant and X-linked	10,000	5-65	40-200
Irregularly inherited	90,000	----	20-900
Recessive	1,100	Very few	Very slow increase
Chromosomal aberration	6,000	0-20	Increases very slightly

mutation that is likely with ionizing radiation (Waldren *et al.*, 1976; ; Thacker, 1986; Evans *et al.*, 1986; Turner *et al.*, 1985; Janatiopour *et al.*, 1988; Bradley *et al.*, 1987; Langlois *et al.*, 1987). Furthermore, cells deficient for the *hgp^{rt}* gene appear *in vivo* to be selected against so that their number decreases with time (Albertini *et al.*, 1988).

1.7.2.1.1 The *hla-A* Method

A new assay suitable for analysis of mutation induction by ionizing radiation has been described (Janatiopour *et al.*, 1988; Turner *et al.*, 1988). This method detects mutants in the *hla* gene family in lymphocytes. Mutant cells are selected or identified using monoclonal antibodies to the proteins HLA-A2 and HLA-A3, the products of commonly occurring alleles found in about half of a human population. Monoclonal antibodies against HLA-A2 or HLA-A3 will, in the presence of complement, kill cells expressing normal versions of these antigens, whereas mutant cells are spared. The latter can be quantitated by scoring colonies or by flow cytometry as described below for the glycophorin A assay. The induction by X-rays of mutations at these loci appears to follow a linear, nonthreshold curve. The doubling dose has been estimated at 1 Gy. Most of the mutants induced by ionizing radiation at the selected *hla-a2* or *hla-a3* loci, or at another *hla* locus about 1 megabases away, but also on the same chromosome, chromosome 6, are deletion and recombinational events. Because this method detects very large mutations it is well suited to studies with ionizing radiation.

1.7.2.1.2 The Glycophorin A Method

This assay uses fluorescently labeled monoclonal antibodies, and the flow cytometric analysis of individual cells, to detect mutations in the allelic forms of glycophorin A, a transmembrane protein of red blood cells (Jensen *et al.*, 1987; Langlois *et al.*, 1987). Glycophorin A exists in an "M" and "N" form that differ from each other by two out of a total of 131 amino acids. These two variants can be independently labeled with antibodies tagged with different fluorochromes that can be differentiated by flow cytometry. Individuals whose red cells display both variants are heterozygous for the M and N alleles. Two kinds of variants are detected: those that have the normal amount of one variant but none of the other (hemizygous loss); and those that are recombinants and have twice the normal content of one variant but none of the other, homozygous or recombination variants (Jensen *et al.*, 1987). These two types of mutants can be distinguished and enumerated by flow cytometry.

The slope of the dose response curves for the production of glycophorin variants among the Japanese A-bomb survivors $0.17 (\pm 0.06) \times 10^{-6}$ per cGy (Langlois *et al.*, 1987). This frequency is similar in general magnitude to that for specific locus mutations in cultured mammalian cells, somatic cells in mice and humans, and mouse germ cells in which frequencies of $0.06-6 \times 10^{-6}$ per cGy have been observed (see Langlois *et al.*, 1987). These values are orders of magnitude larger than for *hgp^{rt}* induced mutations in the lymphocytes of Japanese A-bomb survivors where values of $\sim 0.008 \times 10^{-6}$ per cGy have been reported (Hakoda *et al.*, 1988). It has been suggested that the reason for the relatively small increase in *hgp^{rt}* mutants compared to the frequencies of persistent cytogenetic lesions and glycophorin A variants is that a selective disadvantage exists *in vivo* for mutants lacking the *hgp^{rt}* gene whereas no such selective disadvantage has been observed for the glycophorin A assay (Langlois *et al.*, 1987). Another possibility, of course, is that the functionally hemizygous *hgp^{rt}* locus is closely flanked by essential genes so that the large deletions characteristic of x-ray mutagenesis are lethal and only the smaller ones can produce viable

mutants. Both and other factors could be responsible for these low mutation frequencies.

1.7.2.1.3 The Variant Hemoglobin Method

The frequency of particular, rare hemoglobin variants arising via mutation in red blood cells has also been employed to assess mutagenesis (Papayannopoulou et al., 1976; Stamatodyannopoulos et al., 1982). Cells containing these rare variants are presumed to have arisen as the result of mutation in the stem-cell precursors of the circulating cells. Monoclonal antibodies have been developed that recognize different variants which allows them to be labeled and the mutated cells to be detected by fluorescence cytometry as described for the glycophorin A assay. A serious limitation of this methodology for studies of mutation by ionizing radiation is that it is able to detect only very small mutations that will produce only a change in the amino sequence of beta globin, because larger mutations would disrupt the maturation of these stem cells so that they would not develop into scorable peripheral red cells. This method is further complicated by a fairly high incidence of false positives. (Stamatodyannopoulos et al., 1982).

1.7.2.2 Quantitation of Heritable Mutations

Several assays have been employed to measure heritable mutations in humans, i.e., a mutation present in a germ cell that is passed from parent to offspring. Perhaps the most powerful of these are cytogenic methods.

Another important kind of assay for germ line mutations involves detection and characterization of variant proteins derived from the blood cells of the children of exposed, or control, individuals in a human population. Several well-known, large-scale studies have been carried out on Japanese A-bomb survivors (Neel et al., 1983; Neel et al., 1988). This analysis detects changes created by mutations in a defined set of genes that code for certain proteins. The technique permits mutations to be detected that lead to proteins that display altered electrophoretic properties in 1- or 2-dimensional polyacrylamide gel electrophoresis (O'Farrell, 1975). Thus, this method provides information about mutations in genes from which RNA can be transcribed and which, therefore, may be translated into aberrant proteins.

Although the preceding methodology has provided useful information, it is restricted to the detection of those mutations that change the net charge, molecular weight, and/or conformation of particular proteins so that only a small fraction of the total possible spectrum of mutations can be detected (Neel et al., 1983; Neel et al., 1988). It would appear, therefore, that assays carried out directly on germ or somatic cells offer a substantial advantage over those that require the expression of the mutation in an offspring. This property was demonstrated by the studies of the children of Japanese A-bomb survivors in which the analysis of millions of loci was required to establish mutation rates from heritable changes, whereas samples from only a few directly-affected individuals was sufficient to establish a dose-response curve using the glycophorin A method (Langlois et al., 1987).

1.8 SUMMARY

In either humans or animals, it is not possible to distinguish between a neoplasm that is caused by ionizing radiation and one that occurs

"spontaneously," from some unknown cause. In essence, radiation-induced neoplasms can be recognized in any population by statistical means only; that is, by the identification of an excess frequency above the natural incidence (BEIR III Report, 1980; BEIR V Report, 1990). Neoplasms can be induced in virtually any tissue or organ, however. Some tissues are more sensitive to radiogenic neoplasia in both animals and humans and include bone marrow, breast, lung, and thyroid gland tissues (see Table 1.1). For purposes of a better understanding of risks due to low dose and at low dose rates, it is logical that research should concentrate on these tissues.

Neoplasia develops as a multistage process that proceeds, operationally at least, from normal to neoplastic cells (see Figure 1.1). The first step, *initiation*, reflects a genomic change which produces a transformed cell, that is, one that has altered phenotypic characteristics and can be differentially stimulated to form a focal proliferation of cells. The second step, *promotion*, enhances the formation of a neoplasm in an initiated cell(s) by encouraging proliferation and clonal expansion, that is, the selective accelerated growth of transformed cells. Further steps, included in a process called *progression*, confer new properties on promoted cells and lead to sequential phenotypic changes. The latter are thought to involve additional genetic changes and progressive increases in degrees of malignancy.

A number of biological characteristics can affect radiation oncogenesis (see Table 1.2; Fry and Storer, 1987). In both people and animals, age at exposure is a major determining factor. The general rule is that exposures at earlier ages increase lifetime risks of many types of neoplasia including leukemias, thyroid neoplasia, and mammary neoplasia (BEIR V Report, 1990). Sex of the exposed individual also is a major factor for some types of radiation-induced neoplasms reflecting, at least to some degree, hormonal influences. Associations between increased levels of mammary gland hormone prolactin and increased risk of breast cancer, and between increased levels of thyroid-stimulating hormone and increased risk of thyroid cancer, are well documented (BEIR V Report, 1990; Clifton, 1986; Shellabarger et al., 1986). In some species, hormonal makeup can influence leukemia induction by radiation, as well.

With respect to low-dose radiation tumorigenesis, the same problems of interpretation and significance exist for animal data as for human data. That is, most studies were performed with either high doses of a low-LET radiation, or with lower doses of a high-LET radiation because of its greater biological effectiveness. There is relatively few data on low-dose, low-LET tumor induction. Even for only low-LET exposures, one particular shape of dose-response curve does not appear to fit the data for the induction of several types of tumors (Fry and Storer, 1987). Studies with low-LET radiations have shown that the linear-quadratic or the linear dose-response curves are applicable in given instances, with the latter especially at low dose rates. The effectiveness per unit dose increases with LET. Decreasing the dose rate decreases the effectiveness for low-, but not for high-LET radiations.

Leukemogenesis, a sensitive radiation response in animals and in people, has received considerable attention. A viral etiology had been favored but more recent evidence suggests that radiation-and viral-induced leukemias may be independent processes (Yokoro, 1986). Current models suggest that oncogene activation, chromosomal changes, and growth factor deregulation may be of critical importance in murine radiation leukemogenesis. Radiation-induced acute murine myeloid leukemia cells have consistently shown rearrangements in

chromosome 2 implicating a critical role for the genes in this chromosome in the growth control of myeloid cells. From other studies, cell killing has been identified as a critical factor in the quantitative relationship between dose and tumor induction (Han et al., 1980; Mole 1984; Mole, 1986), and that the effectiveness of an initial low-LET transforming event decreases with protraction of the dose (Han et al., 1980).

Several *in vitro/in vivo* studies of both mammary and thyroid neoplasia have shown that cells, initiated by radiation, appear to be present at a high frequency after radiation exposure, but that the ultimate induction of neoplasms is much less frequent (Adams et al., 1987; Clifton et al., 1986). This observation has suggested that initiation may be a relatively common cellular event, and that additional factors influence the incidence of clinically-apparent neoplasia.

Different animal systems have been used to study lung and respiratory tract neoplasia particularly in reference to the "hot particle problem." The results suggest that doses of highly localized radiation in the lung, whether low- or high-LET, are not more hazardous than uniform exposures (BEIR IV Report, 1988). In fact, localized exposures tend to be less oncogenic probably as a reflection of more localized cell killing.

Studies of radiation-induced skin tumors in the mouse have revealed that, besides acting as an initiating agent, radiation can act to enhance the progression production of squamous carcinomas from benign papillomas (Jaffe et al., 1987). In this process, the product of an oncogene, the protease *transin*, is overexpressed in the malignant, but not in the benign, neoplasms. The over production of transin suggests a role for such an oncogene-associated enzyme in the development of the invasive properties of carcinomas (Matrisian et al., 1986).

Although somatic mutations are the major cellular changes underlying cancer induction, mutagenesis in germ-line cells is also produced by radiation and in general has negative effects on the vitality of offspring. In mice, mutations at specific loci have been demonstrated when either male or female parents are irradiated. In both instances, the frequency of mutations decreased with decreasing dose rate although the decrease was considerably greater for females than for males. Neutrons were appreciable more effective than low-LET radiations.

Techniques based mainly on the analysis of blood cells have been applied to the measurement of somatic mutations in people and new techniques, of considerable sensitivity, are under development. The latter approaches are taking advantage of the methods of genetic engineering, to develop appropriate molecular probes, and innovative electro-optical instrumentation for rapidly scanning large numbers of blood cells. Screening for inherited mutations in children whose parents were irradiated at Hiroshima and Nagasaki, by the method of detecting changes in the proteins in their blood, thus far has not revealed a statistically significant increase. Thus, it would appear that directly measuring the altered properties of germ and somatic cells inherently may offer more sensitivity than techniques that respond to the changes produced in the large number and variety of cells that contribute to the composition of blood in an offspring.

1.9 REFERENCES

- Abrahamson, S., and S. Wolff, "Reanalysis of Radiation Induced Specific Locus Mutations in the Mouse." Nature 264, 715-719, 1976.
- Adams, L. M., S. P. Ethier, and R. L. Ullrich, "Enhanced *in Vitro* Proliferation and *in Vivo* Tumorigenic Potential of Mammary Epithelium from BALB/c Mice Exposed *in Vivo* to γ -Radiation and/or 7,12-Dimethylbenz[a]anthracene," Cancer Research 47, 4425-4431, 1987.
- Albertini, R.J." Studies with T-lymphocytes: An Approach to human Mutagenicity Monitoring," In: Indicators of Genotoxic Exposure, Banbury Report 12, eds., B. A. Bridges, B. E. Butterworth, and I. B. Weinstein, Cold Spring Harbor Laboratory, New York, pp 393-410, 1982.
- Albertini, R.J., L.M. Sullivan, S.K. Berman, C.J. Greene, J.A. Stewart, J.M. Silveira, J.P. O'Neill, "Mutagenicity Monitoring in Humans by Autoradiographic Assay for Mutant T Lymphocytes," Mutation Research 204, 481-492, 1988.
- Anderson, R. E., S. Tokuda, W. L. Williams, and N. L. Warner, "Radiation-Induced Augmentation of the Response of A/J Mice to SaI Tumor Cells," American Journal of Pathology 108, 24-38, 1982.
- Anderson, R. E., J. C. Standefer, and S. Tokuda, "The Structural and Functional Assessment of Cytotoxic Injury of the Immune System with Particular Reference to the Effects of Ionizing Radiation and Cyclophosphamide," British Journal of Cancer 53, Supl. VII, 140-160, 1986.
- Anderson, R. E. and N. L. Warner, "Ionizing Radiation and the Immune Response," Advances in Immunology 24, 215-335, 1976.
- Bair, W. J., "Experimental Carcinogenesis in the Respiratory Tract," in Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 151-167, 1986.
- Banbury Report 28: Mammalian Cell Mutagenesis, eds., M.M. Moore, D. E. DeMarini, F. J. de Serres, and K. R. Tindall, Cold Spring Laboratory Press, 1987.
- Banbury Report 2: Mammalian Cell Mutagenesis: The Maturation of Test Systems, eds., A. W. Hsie, J. P O'Neill, and V. K. McElheny, Cold Spring Laboratory Press, 1979.
- BEIR Report, "Health Effects of Alpha-Emitting Particles in the Respiratory Tract," National Academy of Sciences, National Research Council, Office of Radiation Programs, U. S. Environmental Protection Agency, Washington, D. C., 1976.
- BEIR I Report, "The Effects on Populations of Exposure to Low Levels of Ionizing Radiation," National Academy Press, Washington, D. C., 1972.
- BEIR IV Report, "Health Risks of Radon and Other Internally Deposited Alpha-Emitters," National Academy Press, Washington, D. C., 1988.

- BEIR III Report, "The Effects on Populations of Exposure to Low Levels of Ionizing Radiation," National Academy Press, Washington, D. C., pp. 1-524, 1980.
- BEIR V Report, "Health Effects of Exposure to Low Levels of Ionizing Radiation," National Academy Press, Washington, D.C., 1990.
- Benjamin, S. A., F. F. Hahn, T. L. Chiffelle, B. B. Boecker, and C. H. Hobbs, "Occurrence of Hemangiosarcomas in Beagles with Internally Deposited Radionuclides," Cancer Research 35, 1745-1755, 1975.
- Benjamin, S. A., G. M. Angleton, A. C. Lee, G. K. Miller, B. F. Hamilton, and J. S. Williams, "Carcinogenesis After Irradiation During Development in Beagles: Effect of Age at Exposure." Radiation Research. Proceedings of the Eighth International Congress of Radiation Research, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis, London/Philadelphia, vol. 1, p. 199, 1987.
- Benjamin, S. A., A. C. Lee, G. M. Angleton, W. J. Saunders, G. K. Miller, J. S. Williams, R. D. Brewster, and R. I. Long, "Neoplasms in Young Dogs After Perinatal Irradiation." Journal of the National Cancer Institute 77, 563-571, 1986.
- Boecker, B. B., F. F. Hahn, R. G. Cuddihy, M. B. Snipes, and R. O. McClellan, "Is the Nasal Cavity at Risk from Inhaled Radionuclides?" in Life-Span Radiation Effects Studies in Animals: What Can They Tell Us? CONF-830951, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 564-577, 1986.
- Boice, J. D., Jr., G. Engholm, R. A. Kleinerman, M. Blettner, M. Stovall, H. Lisco, W. C. Moloney, D. F. Austin, A. Bosch, D. L. Cookfair, E. T. Krenmentz, H. B. Latourette, J. A. Merrill, L. J. Peters, M. D. Schultz, H. H. Storm, E. Bjorkholm, F. Pettersson, C. M. J. Bell, M. P. Coleman, P. Fraser, F. E. Neal, P. Prior, N. W. Choi, T. G. Hislop, M. Koch, N. Kreiger, D. Robb, D. Robson, D. H. Thomson, H. Lochmuller, D. V. Fournier, R. Frischkorn, K. E. Kjorstad, A. Rimpela, M-H. Pejovic, V. P. Kirn, H. Stankusova, F. Berrino, K. Sigurdsson, G. B. Hutchinson, and B. MacMahon, "Radiation Dose and Second Cancer Risk in Patients Treated for Cancer of the Cervix," Radiation Research 116, 3-55, 1988.
- Boivin, J. F., and K. O'Brien, "Solid Cancer Risk after Treatment of Hodgkin's Disease," Cancer 61, 2541-2546, 1988.
- Book, S. A., L. S. Rosenblatt, and M. Goldman, "Lifetime Effects of Long-Term Exposures to Strontium-90 and Radium-226 in Beagle Dogs." In, Life-Span Radiation Effects Studies in Animals: What Can They Tell Us? CONF-830951, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 646-659, 1986.
- Bowden, G. T., K. Andrews, and D. Jaffe, "Biological and Molecular Aspects of Radiation Carcinogenesis in Mouse Skin," 37th Annual Radiation Research Society Meeting 109, Abstract, 1989.

- Bradley, W. E. C., J. L. P. Gareau, A. M. Seifert, K. Messing, "Molecular Characterization of 15 Rearrangements Among 90 Human *In Vivo* Somatic Mutants Shows That Deletions Predominate," Molecular, Cellular Biology 7, 956-960, 1987.
- Brent, R. L., D. A. Beckman, and R. P. Jensh, "Relative Radiosensitivity of Fetal Tissues," Advances in Radiation Biology, 12, 239, 1987.
- Brooks, A. L., S. A. Benjamin, R. K. Jones, and R. O. McClellan, "Interaction of ¹⁴⁴Ce and Partial Hepatectomy in the Production of Liver Neoplasms in the Chinese Hamster," Radiation Research 91, 573-588, 1982.
- Burnet, F. M., "Immunological Surveillance in Neoplasia," Transplantation Reviews 7, 3-25, 1971.
- Burns, F. J., and R. E. Albert, "Radiation Carcinogenesis in Rat Skin," in Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 199-214, 1986.
- Cain, G. R., and L. S. Rosenblatt, "Kinetics of Lymphohematopoietic Progenitor Cell Populations in Chronically Irradiated RF/J Mice," Experimental Hematology 13, 1113-1121, 1985.
- Charles, M. W., J. P. Williams, and J. E. Coggle, "Skin Carcinogenesis Following Uniform and Nonuniform Beta Irradiation," Health Physics 55, 399-406, 1988.
- Christov, K., "Radiation-Induced Thyroid Tumors in Infant Rats," Radiation Research 73, 330-339, 1978.
- Clifton, K. H., "Thyroid Cancer: Reevaluation of an Experimental Model for Radiogenic Endocrine Carcinogenesis," in Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 181-198, 1986.
- Clifton, K. H., M. A. Tanner, and M. N. Gould, "Assessment of Radiogenic Cancer Initiation Frequency per Clonogenic Rat Mammary Cell *in Vivo*," Cancer Research 46, 2390-2395, 1986.
- Covelli, V., V. Di Majo, S. Bassani, M. Rebessi, M. Coppola, and G. Silini, "Influence of Age on Life Shortening and Tumor Induction After X-ray and Neutron Irradiation," Radiation Research 100, 348-364, 1984.
- Diamond, L. E., E. W. Newcomb, L. E. McMorro, J. Leon, S. Sloan, I. Guerrero, and A. Pellicer, "In Vivo Activation of Mouse Oncogenes by Radiation and Chemicals," in Radiation Research. Proceedings of the Eighth International Congress of Radiation Research, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis, London/Philadelphia, pp. 532-537, 1987.
- Doria, G., G. Agarossi, and L. Adorini, "Selective Effects of Ionizing Radiation on Immunoregulatory Cells," Immunology Review 65, 23-54, 1982.

- Dungworth, D. L., M. Goldman, D. H. McKelvie, and J. W. Switzer, "Development of a Form of Myelogenous Leukemia in Beagles Exposed Continuously to ⁹⁰Sr." In, Myeloproliferative Disorders in Animals and Man, eds., W. J. Clark, E. B. Howard, and P. L. Hackett, National Technical Information Service, U. S. Department of Commerce, Springfield, Virginia, pp. 272-289, 1970.
- Ethier, S. P. and R. L. Ullrich, "Factors Influencing Expression of Mammary Ductal Dysplasia in Cell Dissociation-Derived Murine Mammary Outgrowths," Cancer Research 44, 4523-4527, 1984.
- Ethier, S. P., L. M. Adams, and R. L. Ullrich, "Morphological and Histological Characteristics of Mammary Dysplasias Occurring in Cell Dissociation-Derived Murine Mammary Outgrowths," Cancer Research 44, 4517-4522, 1984.
- Evans, H. H., J. Mencl, H. Min-Fen, M. Ricanati, C. Sanchez, and J. Hozier, "Locus Specificity in the Mutability of L5178Y Mouse Lymphoma Cells: The Role of Multilocus Lesions." Proceedings of the National Academy of Sciences, USA 83, 4379-4383, 1986.
- Farber, E., "Medical Progress. Chemical Carcinogenesis," New England Journal of Medicine 305, 1379-1389, 1981.
- Farber, E., "Chemical Carcinogenesis. A Biologic Perspective," American Journal of Pathology 106, 271-296, 1982.
- Finkel, M. P., C. A. Reilly, and B. O. Biskis, "Pathogenesis of Radiation and Virus-Induced Bone Tumors," Recent Results in Cancer Research 54, 92-103, 1976.
- Frazier, M. E., T. M. Seed, L. L. Scott, and G. L. Stiegler, "Radiation-Induced Carcinogenesis in Dogs," in Radiation Research. Proceedings of the Eighth International Congress of Radiation Research, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis, London/Philadelphia, pp. 488-493, 1987.
- Fritz, T. E., T. M. Seed, D. V. Tolle, and L. S. Lombard, "Late Effects of Protracted Whole-Body Irradiation of Beagles by Cobalt-60 Gamma Rays," in Life-Span Radiation Effects Studies in Animals: What Can They Tell Us? CONF-830931, eds. R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 116-141, 1986.
- Fry, R. J. M., and R. L. Ullrich, "Combined Effects of Radiation and Other Agents," in Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 437-454, 1986.
- Fry, R. J. M., and J. B. Storer, "External Radiation Carcinogenesis," Advances in Radiation Biology 14, 31-90, 1987.
- Fry, R. J. M., R. D. Ley, D. Grube, and E. Staffeldt, "Studies on the Multistage Nature of Radiation Carcinogenesis." In, Carcinogenesis, ed., E. Hecker, Raven Press, New York, pp. 155-165, 1982.

- Fry, R. J. M., "Experimental Radiation Carcinogenesis: What Have We Learned?" Radiation Research 87, 224-239, 1981.
- Fry, R. J. M., "Relevance of Animal Studies to the Human Experience." In, Radiation Carcinogenesis: Epidemiology and Biological Significance, eds., J. D. Boice, Jr., and J. F. Fraumeni, Jr., Raven Press, New York, pp. 337-346, 1984.
- Gensler, H. L. and G. T. Bowden, "Evidence Suggesting a Dissociation of DNA Strand Scissions and Late-Stage Promotion of Tumor Cell Phenotype," Carcinogenesis 4, 1507-1511, 1983.
- Gilman, E. A., G. W. Kneale, E. G. Knox, and A. M. Stewart, "Recent Estimates of the Risk of Childhood Cancer Following Irradiation of the Fetus." In, Low Dose Radiation, eds., K.F. Baverstock and J. W. Stather, Taylor and Francis, London, pp. 334-339, 1989.
- Goldman, M., and L. K. Bustad, "Biomedical Implications of Radiostrontium Exposure," CONF-710201, Office of Information Services, U. S. Atomic Energy Commission, Washington, D. C., 1972.
- Goldman, M., "Experimental Carcinogenesis in the Skeleton." In, Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 214-231, 1986.
- Goldman, M., L. S. Rosenblatt, and S. A. Book, "Lifetime Radiation Effects Research in Animals: An Overview of the Status and Philosophy of Studies at the University of California-Davis Laboratory for Energy Related Health Research." In, Life-Span Radiation Effect Studies in Animals: What Can They Tell Us? CONF-830951, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 53-65, 1986.
- Gould, M. N., "Radiation Initiation of Carcinogenesis *In Vivo*: A Rare or Common Cellular Event." In, Radiation Carcinogenesis: Epidemiology and Biological Significance, eds., J. D. Boice, Jr., and J. F. Fraumeni, Raven Press, New York, pp. 347-358, 1984.
- Grahn, D., and T. E. Fritz, "Chronic Radiation Injury with Mice and Dogs Exposed to External Whole-Body Irradiation at the Argonne National Laboratory." In, Life Span Radiation Effects Studies in Animals: What Can They Tell Us?, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 14-31, 1986.
- Gray, L. H., "Radiation Biology and Cancer." In, Cellular Radiation Biology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Williams and Wilkins, Baltimore, pp. 7-20, 1965.
- Griffith, W. C., D. L. Lundgren, F. F. Hahn, B. B. Boecker, and R. O. McClellan, "An Interspecies Comparison of the Biological Effects of an Inhaled, Relatively Insoluble Beta Emitter," in Life-Span Radiation Effects Studies in Animals: What Can They Tell Us?, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 501-520, 1986.

- Guerrero, I., P. Calzada, A. Mayer, and A. Pellicer, "A Molecular Approach to Leukemogenesis: Mouse Lymphomas Contain an Activated c-ras Oncogene," Proceedings of the National Academy of Sciences 81, 202-205, 1984a.
- Guerrero, I., A. Villasante, V. Corces, and A. Pellicer, "Activation of a c-K-ras Oncogene by Somatic Mutation in Mouse Lymphomas Induced by Gamma Radiation," Science 255, 1159-1162, 1984b.
- Hahn, F. F., B. B. Boecker, R. G. Cuddihy, C. H. Hobbs, R. O. McClellan, and M. B. Snipes, "Influence of Radiation Dose Patterns on Lung Tumor Incidence in Dogs that Inhaled Beta Emitters," Radiation Research 96, 505-517, 1983.
- Hahn, F. F., B. A. Muggenburg, B. B. Boecker, R. G. Cuddihy, W. C. Griffith, R. A. Guilmette, R. O. McClellan, and J. A. Mewhinney, "Insights into Radionuclide-Induced Lung Cancer in People from Life-Span Studies in Beagle Dogs." In, Life-Span Radiation Effects Studies in Animals: What Can They Tell Us?, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 521-534, 1986.
- Hakoda, M., M. Akiyama, S. Kyoizumi, A.A. Awa, M. Yamakido, M. Otake, "Increased Somatic Cell Mutant Frequency in Atomic Bomb Survivors," Mutation Research 201, 39-48, 1988.
- Han, A., C. K. Hill, and M. M. Elkind, "Repair of Cell Killing and Neoplastic Transformation at Reduced Dose Rates of ^{60}Co γ -rays," Cancer Research 40, 3328-3332, 1980.
- Harvey, E. B., J. D. Boice, Jr., M. Honeyman, and J. T. Flannery, "Prenatal X-ray Exposure and Childhood Cancer in Twins," New England Journal of Medicine 312, 541-545, 1985.
- IAEA Report, Late Biological Effects of Ionizing Radiation, Vol. I and II, International Atomic Energy Agency, Vienna, 1978.
- IAEA Report, Biological and Environmental Effects of Low-Level Radiation, Vol. I and II, International Atomic Energy Agency, Vienna, 1976.
- ICRP Report, "Recommendations of the International Commission on Radiological Protection." Annals of the ICRP, Publication 26, Pergamon Press, 1977.
- ITO, A., M. NAITO, H. WATANABE, and K. YOKORO, "Prolactin and aging: X-irradiated and estrogen-induced rat mammary tumorigenesis." Journal of the National Cancer Institute 123-126, 1984.
- JABLON, S., and H. KATO, "Childhood cancer in relation to prenatal exposure to atomic bomb radiation." Lancet 2, 1000-1003, 1970.
- Jaffe, D. R., and G. T. Bowden, "Ionizing radiation as an initiator in the mouse two-stage model of skin tumor formation." Radiation Research 106, 156-165, 1986.
- Jaffe, D. R., and G. T. Bowden, "Ionizing radiation as an initiator: Effects of proliferation and promotion time on tumor incidence in mice." Cancer Research 47, 6692-6696, 1987.

- Jaffe, D. R., J. F. Williamson, and G. T. Bowden, "Ionizing radiation enhances malignant progression of mouse skin tumors." Carcinogenesis 8, 1753-1755, 1987.
- Janssens, J. P., C. Wittevrongel, J. Van Dan, P. Goddeeris, J. M. Lauwerijns, and W. De Loecker, "Effects of ionizing irradiation on the estradiol and progesterone receptors in rat mammary tumors." Cancer Research 41, 703-707, 1981.
- Janatipour, M., K.J. Trainor, R. Kutlaca, G. Bennett, J. Hay, D.R. Turner, A.A. Morley, "Mutations in Human Lymphocytes Studies by an HLA Selection System," Mutation Research 198, 221-226, 1988.
- Jensen, R.H., W.L. Bigbee, R.G. Langlois, "In Vivo Somatic Mutations in the Glycophorin A Locus of Human Erythroid Cells," Banbury Report 28: Mammalian Cell Mutagenesis, 149-159, 1987.
- Kaul, D. C., "Revised Dosimetry System for Atomic Bomb Survivors." In, Second US-Japan Joint Workshop for Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki, ed., D. J. Thompson, Radiation Effects Research Foundation, Hiroshima, Japan, 1983.
- Kopecky, K. J., and D. L. Preston, "The Impact of the Reassessment of A-Bomb Dosimetry: Dose Estimation Methods and Preliminary Cancer Mortality Findings," Radiation Effects Research Foundation, TR 11-86, 1988.
- Krieg, P., J. Finch, G. Fstenberger, K. Melber, L. M. Matrisian, and G. T. Bowden, "Tumor Promoters Induce a Transient Expression of Tumor-Associated Genes in Both Basal and Differentiated Cells of the Mouse Epidermis," Carcinogenesis 9, 95-100, 1988.
- Kusama, T., and Y. Yoshizawa, "The Carcinogenic Effects of Fetal and Postnatal Radiation in Female Mice," Journal of Radiation Research 23, 290-297, 1982.
- Langlois, R.G., W.L. Bigbee, S. Kyoizumi, N. Nakamura, M.A. Bean, M. Akiyama, R.H. Jensen, "Evidence for Increased Somatic Cell Mutations at the Glycophorin A Locus in Atomic Bomb Survivors." Science 236, 445-448, 1987.
- Le, J., and J. Vilcek, "Tumor Necrosis Factor and Interleukin 1: Cytokines with Multiple Overlapping Biological Activities." Laboratory Investigation 56, 234-248, 1987.
- Lee, W., R. P. Chiacchierini, B. Schlein, and N. C. Telles, "Thyroid Tumors Following ¹³¹I or Localized X-irradiation to the Thyroid and Pituitary Glands in Rats," Radiation Research 92, 307-319, 1982.
- Little, J. B., "Influence of Noncarcinogenic Secondary Factors on Radiation Carcinogenesis," Radiation Research 87, 240-250, 1981.
- Lotem, J. and L. Sachs, "In Vivo Control of Differentiation of Myeloid Leukemic Cells by Cyclosporine A and Recombinant Interleukin-1 Alpha," Blood 71, 1595-1601, 1988.

- Lotem, J. and L. Sachs, "Indirect Induction of Differentiation of Normal and Leukemic Myeloid Cells by Recombinant Interleukin 1," Leukemia Research 13, 13-21, 1989.
- Lyon, M. F., R. J. S. Phillips, and G. Fisher, "Dose-Response Curves for Radiation-Induced Mutations in Mouse Oocytes and Their Interpretation." Mutation Research 63, 161-***, 1979.
- Maisin, J. R., A. Wambersie, G. B. Gerber, G. Mattelin, M. Lambiet-Collier, and J. Gueulette, "The Effects of a Fractioned Gamma Irradiation on Life Shortening and Disease Incidence in BALB/c Mice," Radiation Research 94, 359-373, 1983a.
- Maisin, J. R., A. Wambersie, G. B. Gerber, J. Gueulette, G. Mattelin, and M. Lambiet-Collier, "Life shortening and disease incidence in BALB/c mice following single d(50)-Be neutron or gamma exposure." Radiation Research 94, 374-389, 1983b.
- Matrisian, L. M., G. T. Bowden, P. Krieg, G. Furstenberger, J. P. Briand, P. Leroy, and R. Breathnach, "The mRNA Coding for the Secreted Protease Transin is Expressed More Abundantly in Malignant than in Benign Tumors," Proceedings of the National Academy of Sciences 83, 9413-9417, 1986.
- McClellan, R. O., B. B. Boecker, F. F. Hahn, and B. A. Muggenburg, "Lovelace ITRI Studies on the Toxicity of Inhaled Radionuclides in Beagle Dogs," Life-Span Radiation Effects Studies in Animals: What Can They Tell Us?, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 74-96, 1986.
- Miller, S. C., W. S. S. Jee, J. M. Smith, and T. J. Wronski, "Tissue Characteristics of High- and Low-Incidence Plutonium-Induced Osteogenic Sarcoma Sites in Life-Span Beagles," Life-Span Radiation Effects Studies in Animals: What Can They Tell Us? CONF-830951, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 286-298, 1986.
- Mole, R. H. "Radiation-Induced Acute Myeloid Leukemia in the Mouse: Experimental Observations *in Vivo* with Implications for Hypotheses about the Basis of Carcinogenesis," Leukemia Research 10, 859-865, 1986.
- Mole, R. H., "Dose-Response Relationships." In, Radiation Carcinogenesis: Epidemiology and Biological Significance, eds., J. D. Boice, Jr. and J. F. Fraumeni, Jr., Raven Press, New York, pp. 403-419, 1984.
- Monson, R. R. and B. MacMahon, "Prenatal X-Ray Exposure and Cancer in Children." In, Radiation Carcinogenesis: Epidemiology and Biological Significance, eds., J. D. Boice, Jr., and J. F. Fraumeni, Jr., Raven Press, New York, pp. 97-105, 1984.
- Muirhead, C. R., and S. C. Darby, "Modelling the Relative and Absolute Risks of Radiation-Induced Cancers," Journal of the Royal Statistical Society A. 150, Part 2, 83-118, 1987.

- Muirhead, C. R., and S. C. Darby, "Relative and Absolute Risk Models for Cancer Mortality in Ankylosing Spondylitis Patients," In, Low Dose Radiation, eds., K.F. Baverstock and J. W. Stather, Taylor and Francis, London, pp. 162-170, 1989.
- Mulcahy, R. T., M. N. Gould, and K. H. Clifton, "Radiogenic Initiation of Thyroid Cancer: A Common Cellular Event," International Journal of Radiation Biology 45, 419-426, 1984.
- Muller, H. J., "Artificial Transmutation of the Gene." Science 66, 84-87, 1927.
- NCRP Report, Induction of Thyroid Cancer by Ionizing Radiation, National Council on Radiation Protection and Measurements, Bethesda, Maryland, 1985.
- NCRP Report, Influence of Dose and its Distribution in Time on Dose-Response Relationships for Low-LET Radiations, National Council on Radiation Protection and Measurements, Bethesda, Maryland, 1980.
- NCRP Report, Comparative Carcinogenicity of Ionizing Radiation and Chemicals, National Council on Radiation Protection and Measurements, Bethesda, Maryland, 1989.
- NCRB Report, "Health Effects Models Developed from the 1988 UNSCEAR Report," Strather, J. W., C. R. Muirhead, A. A. Edwards, J. D. Harrison, D. C. Lloyd, and N. R. Wood, National Radiation Protection Board Report NRPB-R226, Chilton, Didcot, Oxon, United Kingdom, 1988.
- Neel, J.V., C. Satoh, K. Goriki, J. Asakawa, M. Fujita, N. Takahashi, T. Kageoka, R. Hazama, "Search for Mutations Altering Protein Charge and/or Function in Children of Atomic Bomb Survivors: Final Report." American Journal of Human Genetics 42, 663-676, 1988.
- Neel, J.V., H. Mohrenweiser, S. Hanash, et al. "Biochemical Approaches to Monitoring Human Populations for Germinal Mutation Rates," In: Electrophoresis; Utilization of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk, eds., F. J. de Serres, and W. Sheridan, Plenum Press, New York, pp 71-93, 1983.
- Newcomb, E. W., R. Binari, and E. Fleissner, "A Comparative Analysis of Radiation- and Virus-Induced Leukemias in BALB/c Mice," Virology 140, 102-112, 1985.
- Nolibe, D., R. Masse, and J. Lafuma, "The Effect of Neonatal Thymectomy on Lung Cancers Induced in Rats by Plutonium Dioxide," Radiation Research 87, 90-99, 1981.
- Nowell, P. C. and C. M. Croce, "Chromosomal Approaches to Oncogenes and Oncogenesis," FASEB J. 2, 3054-3060, 1988.
- O'Farrell, P. H., "High Resolution Two Dimensional Electrophoresis of Proteins," Journal of Biological Chemistry, 250 4007-4021, 1975.

- Neel, J. V., B. B. Rosenblum, C. F. Sing, M. M. Skolnick, S. M. Hanash, S. Sternberg. "Adapting Two-Dimensional Gel Electrophoresis to the Study of Human Germline Mutations Rates," In: Methods and Applications of Two Dimensional Gel Electrophoresis of Proteins, eds., J. E. Celis, and R. Bravo, Academic Press, New York, p. 259, 1984.
- Oppenheim, B. E., M. L. Griem, and P. Meier, "The Effects of Diagnostic X-Ray Exposure on the Human Fetus: An Examination of the Evidence," Radiology 114, 529-534, 1975.
- Papayannopoulou, T., T.C. McGuir, T. Lim, G. Stamatoyannopoulos, "Identification of Haemoglobin S in Red Cells and Normoblasts Using Fluorescent anti-HB S Antibodies," British Journal of Haematology 34, 25-31, 1976.
- Preston, D. L., and D. A. Pierce, "The Effect of Changes in Dosimetry on Cancer Mortality Risk Estimates in the Atomic Bomb Survivors." Radiation Effects Research Foundation TR 9-87, Hiroshima, Japan, 1987.
- Purtilo, D. T. and K. Sakamoto, "Immunodeficiency as a Factor in Lymphomagenesis," Perspectives in Pediatric Pathology 8, 181-191, 1984.
- Reif, A. E. and W. E. Triest, "Vaccination Against Strontium-90-Induced Bone Tumors," Journal of the National Cancer Institute 71, 545-552, 1983.
- Rice, J. M., "Fetal Susceptibility to Viral and Chemical Carcinogens," Laboratory Investigation 58, 1-4, 1988.
- Roesch, W. J. (Ed.), "Final Report of the US-Japan Reassessment of the Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki." Radiation Effects Research Foundation, Hiroshima, Japan, 1987.
- Ron, E., B. Modan, and J. D. Boice, Jr., "Mortality after Radiotherapy for Ringworm of the Scalp." American Journal of Epidemiology 127, 713-725, 1988.
- Sanders, C., K. E. McDonald, and J. A. Mahaffey, "Lung Tumor Response to Inhaled Pu and its Implications for Radiation Protection," Health Physics 55, 455-462, 1988.
- Sasaki, S., "Consequences of Prenatal Irradiation in Mice: Carcinogenesis and CNS Damage as a Basis for Human Risks," Radiation Research. Proceedings of the Eighth International Congress of Radiation Research, Vol. 2, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis London/Philadelphia, pp. 652-657, 1987.
- Sawey, M. J., A. T. Hood, F. J. Burns, and S. J. Garte, "Activation of c-myc and c-K-ras Oncogenes in Primary Rat Tumors Induced by Ionizing Radiation," Molecular and Cellular Biology 7, 932-935, 1987.
- Searle, A.G., "Mutation Induction in Mice." In, Advances in Radiation Biology 4, eds., J. T. Lett, H. Adler, and M. R. Zelle, Academic Press, New York, pp. 131-207, 1974.

- Seed, T. M., L. V. Kaspar, T. E. Fritz, and D. V. Tolle, "Cellular Responses in Chronic Radiation Leukemogenesis." In, Carcinogenesis, eds., E. Huberman and S. H. Barr, Raven Press, New York, pp. 363-377, 1985.
- Seemayer, T. A. and W. K. Cavenee, "Biology of Disease; Molecular Mechanisms of Oncogenesis," Laboratory Investigation 60, 585-599, 1989.
- Shellabarger, C. J., J. P. Stone, and S. Holtzman, "Experimental Carcinogenesis in the Breast." In, Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 169-180, 1986.
- Shellabarger, C. J., J. P. Stone, and S. Holtzman, "Effect of Interval Between Neutron Radiation Diethylstilbestrol on Mammary Carcinogenesis in Female ACI Rats," Environmental Health Perspectives 50, 227-232, 1983.
- Shimizu, Y., H. Kato, W. J. Schull, D. L. Preston, S. Foujita, and D. A. Pierce, "Comparison of Risk Coefficients for Site-Specific Cancer Mortality Based on the DS86 and T65DR Shielded Kerma and Organ Doses," Radiation Effects Research Foundation, Life Span Study Report 11, Part 1, RERF TR 12-87, Hiroshima, Japan, 1987.
- Shimizu, Y., H. Kato, and W. J. Schull, "Cancer Mortality in the Years 1950-1985 Based on the Recently Revised Doses (DS86)," Radiation Effects Research Foundation, Life Span Study Report 11, Part 2, TR 5-88, Hiroshima, Japan, 1988.
- Shiono, P. H., C. S. Chung, and N. C. Myrianthopoulos, "Preconception Radiation, Intrauterine Diagnostic Radiation, and Childhood Neoplasia," Journal of the National Cancer Institute 65, 681-686, 1980.
- Shore, R. E., E. Woodard, N. Hildreth, P. Dvoretzky, L. Hemplemann, and B. Pasternack, "Thyroid Tumors Following Thymus Irradiation," Journal of the National Cancer Institute, 74, 1177-1184, 1985.
- Shore, R. E., N. Hildreth, E. Woodard, P. Dvoretzky, L. Hemplemann, and B. Pasternack, "Breast Cancer Among Women Given X-Ray Therapy for Acute Postpartum Mastitis," Journal of the National Cancer Institute, 77, 689-696, 1986.
- Sikov, M. R., "Carcinogenesis Following Prenatal Exposure to Radiation," Biological Research in Pregnancy 2, 159-167, 1981.
- Silver, A., J. Boulwood, G. Breckon, W. K. Masson, J. Adam, and R. Cox, "Studies on an Initiating Event for Radiation Myeloid Leukemogenesis," 37th Annual Radiation Research Society Meeting, 108, Abstract, 1989.
- Silver, A. R., W. K. Masson, G. Breckon, and R. Cox, "Preliminary Molecular Studies on Two Chromosome 2 Encoded Genes, c-abl and Beta 2M, in Radiation-Induced Murine Myeloid Leukaemias," International Journal of Radiation Biology 53, 57-63, 1988.
- Silver, A., G. Breckon, W. K. Masson, D. Malowany, and R. Cox, "Studies on Radiation Myeloid Leukemogenesis in the Mouse," in Radiation Research. Proceedings of the Eighth International Congress of Radiation Research.

- Vol. 2, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis, London/Philadelphia, pp. 494-500, 1987.
- Solleveld, H. A., M. J. Van Zwieten, J. J. Broerse, and C. F. Hollander, "Effects of X-Irradiation, Ovariohysterectomy and Estradiol-17 Beta on Incidence, Benign/Malignant Ratio and Multiplicity of Rat Mammary Neoplasms--A Preliminary Report," Leukemia Research 10, 755-759, 1986.
- Stamatodyannopoulos, G., P.E. Nute, "De Novo Mutations Producing Unstable Hbs or HbsM.II: Direct Estimates of Minimum Nucleotide Mutation Rates in Man," Human Genetics 60, 181-188, 1982.
- Stewart, A., J. Webb, and D. Hewitt, "A Survey of Childhood Malignancies," British Medical Journal 1, 1495-1508, 1958.
- Stewart, A., and G. W. Kneale, "Radiation Dose Effects in Relation to Obstetric X-Rays and Childhood Cancers," Lancet 1, 1185, 1970.
- Stone, J. P., S. Holtzman, and C. J. Shellabarger, "Synergistic Interactions of Various Doses of Diethylstilbestrol and X-Irradiation on Mammary Neoplasia in Female ACI Rats," Cancer Research 40, 3966-3972, 1980.
- Storer, J. B., T. J. Mitchell, and R. J. M. Fry, "Extrapolation of the Relative Risk of Radiogenic Neoplasms Across Mouse Strains and to Man," Radiation Research 114, 331-353, 1988.
- Storer, J. B., T. J. Mitchell, and R. L. Ullrich, "Causes of Death and their Contribution to Radiation-Induced Life Shortening in Intact and Ovariectomized Mice," Radiation Research 89, 618-643, 1982.
- Storer, J. B., "Carcinogenic Effects: An Overview." In, Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 11-22, 1986.
- Storer, J. B., L. J. Serrano, E. B. Darden, M. C. Jernigan, and R. L. Ullrich, "Life Shortening in RFM and BALB/c Mice as a Function of Radiation Quality, Dose and Dose Rate," Radiation Research 78, 122-161, 1979.
- Stutman, O., "Natural Antitumor Resistance in Immune-deficient Mice," Experimental Cell Biology 52, 30-39, 1984.
- Stutman, O., "Immunological Surveillance and Cancer," in The Handbook of Cancer Immunology, H. Waters, ed., Garland STPM Press, New York, pp. 1-25, 1981.
- Tates, A.D., L.F. Bernini, A.T. Natarajan, J.S. Ploem, N.P. Verwoerd, J. Cole, M.H.L. Green, C.F. Arlett, P.N. Norris, "Detection of Somatic Mutants in Man: "HPRT Mutations in Lymphocytes and Hemoglobin Mutations in erythrocytes," Mutation Research 213, 73-82, 1989.
- Taylor, G. N., C. W. Mays, M. E. Wrenn, L. Shabestari, and R. D. Lloyd, "Incidence of Liver Tumors in Beagles with Body Burdens of ²³⁹Pu or ²⁴¹Am," in Life-Span Radiation Effects Studies in Animals: What Can They Tell Us? eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 268-285, 1986.

- Technologies for Detecting Heritable Mutations in Human Beings. 1986, U.S. Congress, Office of technology Assessment, OTA-H-298, Washington, D.C., Government Printing Office
- Terzaghi, M., P. Nettesheim, and L. Riester, "Effect of Carcinogen Dose on the Dynamics of Neoplastic Development in Rat Tracheal Epithelium," Cancer Research 42, 4511-4518, 1982a.
- Terzaghi, M., A. Klein-Szanto, and P. Nettesheim, "Effect of the Promoter 12-O-Tetradecanoylphorbol-13-acetate on the Evolution of Carcinogen-Altered Cell Populations in Tracheas Initiated with 7,12-Dimethylbenz(a)anthracene," Cancer Research 43, 1461-1466, 1982b.
- Terzaghi-Howe, M. and C. McKeown, "Inhibition of Carcinogen-Altered Rat Tracheal Epithelial Cells by Normal Epithelial Cell-Conditioned Medium," Cancer Research 46, 917-921, 1986.
- Terzaghi-Howe, M., "Inhibition of Carcinogen Altered Rat Tracheal Epithelial Cell Proliferation by Normal Epithelial Cells *in Vivo*," Carcinogenesis 8, 145-150, 1987.
- Terzaghi-Howe, M., "Interactions Between Cell Populations Influence Expression of the Transformed Phenotype in Irradiated Rat Tracheal Epithelial Cells," 37th Annual Radiation Research Society Meeting 109, Abstract, 1989.
- Thacker, J., "The Nature of Mutants Induced by Ionizing Radiation in Cultured Hanster Cells. III. Molecular characterization of the HPRT-deficient Mutants Induced by Gamma Rays or Alpha Particles Showing that the Majority have Deletions of All or Part of the HPRT Gene.", Mutation Research 160, 267-275, 1986.
- Thompson, R. C., F. T. Cross, G. E. Dagle, J. F. Park, and C. L. Sanders, "DOE Life-Span Radiation Effects Studies at Pacific Northwest Laboratory." In, Life-Span Radiation Effects Studies in Animals: What Can They Tell Us? CONF-830951, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 66-73, 1986.
- Thompson, R. C., and J. A. Mahaffey, Eds. Life-Span Radiation Effects Studies in Animals. What Can They Tell Us? Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., 1986.
- Thomson, J. F., and D. Grahn, "Life Shortening in Mice Exposed to Fission Neutrons and Gamma Rays," VII. Effects of 60 Once-Weekly Exposures," Radiation Research 115, 347-360, 1980.
- Thomson, J. F., F. S. Williamson, D. Grahn, and E. J. Ainsworth, "Life Shortening in Mice Exposed to Fission Neutrons and Gamma Rays. I. Single and Short-Term Fractioned Exposures," Radiation Research 86, 559-572, 1981.
- Turner, D.R., A.A. Morley, M. Haliandros, R. Kutlaca, B. J. Sanderson, "In Vivo Somatic Mutations in Human Lymphocytes Frequently Result From Major Gene Alterations," Nature 315, 343-345, 1985.

- Turner, D.R., S.A. Grist, M. Janatipour, A.A. Morley, "Mutations in Human Lymphocytes Commonly Involve Gene Duplication and Resemble Those Seen in Cancer Cells," Proceedings of the National Academy Science 85, 3189-3192, 1988.
- Ullrich, R. L., "Tumor Induction in BALB/c Female Mice After Fission Neutron or Gamma Irradiation," Radiation Research 93, 506-515, 1983.
- Ullrich, R. L., "Tumor Induction in BALB/c Mice After Fractionated or Protracted Exposures to Fission-Spectrum Neutrons," Radiation Research 97, 587-597, 1984.
- Ullrich, R. L., "Carcinogenesis in Mice After Low Doses and Dose Rates," in Radiation Biology in Cancer Research, eds., R. E. Meyn and H. R. Withers, Raven Press, New York, pp. 309-319, 1980.
- Ullrich, R. L., M. C. Jernigan, L. C. Satterfield, and N. D. Bowles, "Radiation Carcinogenesis: Time-Dose Relationships," Radiation Research 111, 179-184, 1987.
- Ullrich, R. L., "The Rate of Progression of Radiation-Transformed Mammary Epithelial Cells is Enhanced After Low-Dose Rate Neutron Irradiation," Radiation Research 105, 68-75, 1986.
- UNSCEAR Report, "Sources, Effects, and Risks of Ionizing Radiation; 1988 Report to the General Assembly (with Annexes), United Nations, New York, 1988.
- Upton, A. C., R. E. Albert, F. J. Burns, and R. E. Shore, eds., Radiation Carcinogenesis, Elsevier Science Publishing Co., Inc., New York, 1986.
- Upton, A. C., "Biological Aspects of Radiation Carcinogenesis," in Radiation Carcinogenesis: Epidemiology and Biological Significance, eds., J. D. Boice, Jr., and J. F. Fraumeni, Jr., Raven Press, New York, pp. 9-19, 1984.
- Upton, A. C., "Historical Perspectives on Radiation Carcinogenesis," in Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier Science Publishing Co., Inc., New York, pp. 1-10, 1986.
- Upton, A. C., M. L. Randolph, and J. W. Conklin, "Late Effects of Fast Neutrons and Gamma-Rays in Mice as Influenced by Dose Rate of Irradiation: Induction of Neoplasia," Radiation Research 41, 467-491, 1970.
- USNRC Report, "Health Effects Models for Nuclear Power Plant Accident Consequence Analysis," NUREG/CR-4214, U.S. Nuclear Regulatory Commission, Washington, D.C. 1989.
- USNRC Report, "Reactor Safety Study: An Assessment of Accidental Releases." In, U.S. Commercial Nuclear Power Plants WASH-1400, U.S. Nuclear Regulatory Commission, Washington, D. C., 1975.
- Waldren, C., L. Correll, M. A. Sognier, and T. T. Puck, "Measurement of Low Levels of X-Ray Mutagenesis in Relation to Human Disease." Proceedings of the National Academy of Sciences USA 83, 4839-4843, 1986.

- Waldren, C., C. Jones, and T. T. Puck, "Measurement of Mutagenesis in Mammalian Cells." Proceedings of the National Academy of Sciences USA 76, 1358-1362, 1979.
- Watanabe, H., M. N. Gould, P. A. Mahler, R. T. Mulcahy, and K. H. Clifton, "The Influence of Donor and Recipient Age and Sex on the Quantitative Transplantation of Monodispersed Rat Thyroid Cells," Endocrinology 112, 172-177, 1983b.
- Watanabe, H., M. A. Tanner, F. E. Domann, M. N. Gould, and K. H. Clifton, "Inhibition of Carcinoma Formation and of Vascular Invasion in Grafts of Radiation-Initiated Thyroid Clonogens by Unirradiated Thyroid Cells," Carcinogenesis 9, 1329-1335, 1988.
- Watanabe, H., M. N. Gould, D. P. Rose, and K. H. Clifton, "The Effect of Thyrotropin (TSH) Levels on Follicular Cluster Formation from Grafted Monodispersed Rat Thyroid Cells," Proceedings of the Society of Experimental Biology and Medicine 173, 21-26, 1983a.
- Watanabe, H., A. Ito, and F. Hirose, "Experimental Carcinogenesis in the Digestive and Genitourinary Tracts," in Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 233-244, 1986.
- Wrenn, M. E., G. N. Taylor, W. Stevens, C. W. Mays, W. S. S. Jee, R. D. Lloyd, D. R. Atherton, F. W. Bruenger, S. C. Miller, J. M. Smith, L. R. Shabestari, L. A. Woodbury, and B. J. Stover, "DOE Life-Span Radiation Effects Studies in Experimental Animals at University of Utah Division of Radiobiology." In Life-Span Radiation Effects Studies in Animals: What Can They Tell Us?, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 32-52, 1986a.
- Wrenn, M. E., G. N. Taylor, W. Stevens, C. W. Mays, W. S. S. Jee, R. D. Lloyd, D. R. Atherton, F. W. Bruenger, D. B. Kimmel, S. C. Miller, L. R. Shabestari, J. M. Smith, L. A. Woodbury, and B. J. Stover, "Summary of Dosimetry, Pathology, and Dose Response for Bone Sarcomas in Beagles Injected with Radium-226," in Life-Span Radiation Effects Studies in Animals: What Can They Tell Us?, eds., R. C. Thompson and J. A. Mahaffey, Office of Scientific and Technical Information, U. S. Department of Energy, Washington, D. C., pp. 253-267, 1986b.
- Yokoro, K., C. Sumi, A. Ito, K. Hamada, K. Kanda, and T. Kobayashi, "Mammary Carcinogenic Effect of Low-Dose Fission Radiation in Wistar/Furth Rats and its Dependency on Prolactin," Journal of the National Cancer Institute 64, 1459-1466, 1980.
- Yokoro, K., "Experimental Radiation Leukemogenesis in Mice," in Radiation Carcinogenesis, eds., A. C. Upton, R. E. Albert, F. J. Burns, and R. E. Shore, Elsevier, New York, pp. 137-150, 1986.
- Yoshimoto, Y., H. Kato, and W. J. Schull, "Risk of Cancer Among Children Exposed *in Utero* to A-Bomb Radiations," Lancet 2, 665-669, 1988.

Yuspa, S. H., and M. C. Poirier, "Chemical Carcinogenesis: From Animal Models to Molecular Models in One Decade," Advances in Cancer Research 50, 25-69, 1988.

Task 2

"Mutation, Neoplastic Transformation, and Tumor Formation" *Molecular Mechanisms*

2.1 INTRODUCTION

In this Task, the present state of knowledge of the cellular/molecular basis for tumorigenesis is reviewed with particular emphasis on radiation-induced changes.

The major processes that are associated with the multiple steps in tumorigenesis are shown schematically in Fig. 2.1. It is generally recognized that cancer is a cellular disease that may arise from the transformation of a single precursor cell even though substantial evidence indicates that cells in addition to the progenitor cell from which a cancer develops may be involved in the expression of the neoplastically transformed state. Neoplasms become evident usually when the successive steps of *initiation*, *promotion*, and *progression* have occurred. More than one cell type, normal and/or abnormal, may be involved in this sequence. It is likely that the properties of the cells that constitute a neoplasm are altered during these successive steps so that, despite the clonal origin of a tumor, the population of cells of which it is constituted may be sufficiently heterogeneous to make difficult at best the identification of the molecular changes that initiated the process.

The energetics of the absorption of ionizing radiation suggest the likelihood that its action will have features that are qualitatively different from those of other cancer-inducing etiological agents. These features are likely to be relatively unique and to apply at least to the initiation step. However, radiation-induced cancers are indistinguishable from those produced by other agents. Consequently, one can expect that considerable overlap exists in the successive changes, which make up the multiple steps that culminate in cancer, due to different causative agents. This overlap is evident even at a cellular level as the discussion to follow will make evident (see, Tannock and Hill, 1987; Furth and Greaves, 1989; Seemayer and Cavenee, 1989).

2.2 MUTAGENESIS AND CANCER

A neoplastically transformed cell is one that has suffered heritable change. Indeed, the evidence is convincing that mutation is a primary cause of cancer (Miller & Miller, 1971; Ames et al., 1973; National Research Council, 1983; Office of Technology Assessment, 1986). Evidence for the involvement of mutation in cancer includes: 1) most carcinogens are also mutagens; 2) in their active form, most carcinogens can bind to and damage DNA; 3) specific mutations are found in many specific tumors; and 4) tumor formation is often associated with the activation of oncogenes or the inactivation of anti-oncogenes (see Section 2.3). In reference to point 4), changes in oncogene activity can be caused by a variety of genetic changes including mutations, deletions, translocations, amplifications in the number of gene copies, and the changes that give rise to aneuploidy. Although all of the foregoing may be induced by different etiological agents, it is likely that the relative proportions will vary depending upon the agent. In addition, people who have an inherited defect in their ability to repair damage to their DNA may have an increased susceptibility to cancer. Aside from defects in repair, familial cancers have

Fig. 2.1 Schematic representation of the changes associated with the induction of neoplasia by a DNA damaging agent like radiation. In the instance of familial cancer, ordinarily one of the gametes will have a mutated (-) gene.

been identified whose incidence depends on certain inherited factors. As discussed in Sections 2.3 & 2.5, the latter kinds of tumors have been important in illuminating the cell/molecular biology of neoplasia.

Although mutations arising from damage to DNA can initiate cancer, alterations in other kinds of molecules, such as RNA or regulatory proteins, appear to play a role as well (Miller and Miller, 1971; Lijinsky, 1989). Further, non-mutagenic alterations to DNA--such as the methylation of specific cytosine residues that can influence the regulation of some genes--may be involved in initiation and cell progression (Jones, 1986). Along with many other physical and chemical agents, ionizing radiation can produce mutations in germ and somatic cells and can, thereby, cause cancer (see BEIR III Report, 1980; Doll and Peto, 1981; Sankaranarayanan, 1982; BEIR V Report, 1990).

In broad terms, cancers may be thought of as belonging to one of four categories all of which may involve mutations (see Knudson, 1985): 1) cancers that are due to environmental agents; 2) cancers that are due to hereditary factors; 3) cancers that are due to both environmental and hereditary factors; or 4) cancers that appear "spontaneously", i.e., the specific cause is unidentified. It appears likely that the interaction of environmental and hereditary factors may be a major cause of human neoplasia. But even though an entire population may be exposed to a causative agent, cancers appear only in a small proportion of individuals. The genetic factors that may control susceptibility are beginning to be understood (see Knudson, 1985; Environmental Health Institute, 1989); still, a complete knowledge of what the factors are, how they affect cells, and to what degree they are influenced particularly by radiation, is needed.

2.2.1 Inherited Susceptibility

Many individuals are born with various kinds of mutations that predispose them to different diseases including cancer (see BEIR III Report, 1980; German, 1983; Environmental Health Institute, 1989; Cavenee et al., 1983; Vijayalaxmi et al., 1983; Knudson, 1986; Pearson and Rowley, 1985). Somatic mutations, including those resulting from unrepaired or misrepaired DNA damage, may be acquired pre- or postnatally. As mentioned above, the combination of heritable predisposition plus subsequent mutation caused by exposure to radiation or other etiological agents may result in cancer. In addition to repair deficiencies, examples of predispositions are: variations in the activity of the aryl hydrocarbon hydroxylases, cytochrome P-450 mixed function oxidases (i.e., enzyme systems that may activate or deactivate polycyclic aromatic hydrocarbons and other indirectly acting mutagens/carcinogens); deficiencies in the immune system, which may prevent the recognition and destruction of incipient cancer cells; and/or inheritance of a mutation in one allele of an anti-oncogene (see Sections 2.3 & 2.5).

Mutations in cells that are fully differentiated, and therefore no longer able to divide, may be essentially innocuous. Such cells probably do not play a role in transformations leading to neoplasia unless they are first induced to revert to an undifferentiated state. On the other hand, viable mutations in stem cells, or in the cells constituting a cell-renewal system, are likely to be expressed and to be deleterious (see Comings, 1973), unless they are selected against, because the mutation places them at a growth disadvantage. Thus, a neoplasm could take the form of an uncontrolled increase in a stem-cell population, and/or a decrease in a normal rate of differentiation, leading to an

abnormal increase in the number of mitotically active cells of the type in question. In addition, by itself increased cell division can lead to an increased risk of expressing an initiated state.

2.2.2 Repair/Misrepair

Defective repair, either in the form of a diminished capacity for accurate repair or through inaccurate repair processes, can lead to heritable alterations (mutations) involving genes responsible for the control of cell proliferation (see Friedberg, 1985; Cleaver, 1989). It has been amply demonstrated that an individual inherits a capacity for repair and that defective repair can be associated with neoplasia (see Swift et al., 1987). Following exposure to a cancer-inducing agent, a mutant cell occasionally may be created with the potential to form a tumor. The kinds of genetic changes that are known to be involved are described in Section 3.2. Gene mutations, nonlethal chromosomal deletions or rearrangements, gene amplifications, and aneuploidies are included among such changes. Tumors that appear "spontaneously" also may be due to a misrepair if an error-correcting mechanism had malfunctioned. Spontaneous tumors may result from endogenous DNA damage resulting, for example, from normal oxidative processes. The latter are estimated to produce in the genome of human cells 1×10^4 oxidations/day (Ames, 1989).

An awareness of the importance of repair processes first came from studies in radiobiology. Repair processes involving DNA are known to play prominent roles in influencing the phenotypic outcome of exposure to physical, chemical, or viral agents. At a molecular level, processes such as prereplicative repair (repair before the damaged DNA is replicated) can reinsert the correct base sequences--as exemplified by the steps in excision repair, i.e., incision, excision, resynthesis, and ligation--by using the undamaged strand of DNA as a template for faithful synthesis. Excision repair can be error-free, hence a mutation would not result. There is evidence that some recovery processes allow mammalian cells to tolerate damage that has not been removed by, for example, a process called recombinational repair. Such processes, which may be postreplicative and involve DNA recombination, may bypass adducts and other types of lesions so that they remain in the genome as potential sources of mutation. Cells, which are deficient in this type of repair, may be hypomutable (Stamato et al., 1981). Still, other processes mend breaks in DNA, processes that can result in the restitution of a chromosome to its original configuration, or to an altered chromosomal rearrangement that may allow a cell to survive and, therefore, to express a mutation.

Much of our knowledge about molecular mechanisms of DNA repair and mutagenesis comes from the application of genetic, biochemical, and molecular studies to microorganisms like the bacterium *E. coli* and the yeast *S. cerevisiae* (Friedberg, 1985; Hanawalt et al., 1979). Although these microorganisms have provided useful models for experimental design, DNA repair systems in mammalian cells have proved to be more complex and, therefore, less well understood. For example, in mammalian cells it is not yet clear that repair systems are inducible in contrast to micro-organisms where enhanced expression of specific repair genes in response to DNA damage or replicative arrest is well established (Walker, 1985). In *E. coli*, mutagenesis is not simply a passive process but requires the involvement of the products of particular genes (Walker, 1985). That is, specific enzymatic processing of DNA is required for a mutation to be effected.

Repair effectiveness in mammalian cells may be controlled in other ways, however. For example, repair may be more effective in transcriptionally active DNA as opposed to regions of the genome that are nontranscribed, and the transcribed strand may be preferentially repaired (Hanawalt et al., 1986; Mellon et al., 1987). It should be noted that such preferential repair has not as yet been demonstrated for the principal kinds of lesions caused by ionizing radiation.

Even in repair-competent cells, most genotoxic treatments that result in proportions of surviving cells from 10 to 100 percent, lead to the deposition of many more lesions of various types than are needed apparently to effect lethality. For example, as many as $\sim 5 \times 10^5$ pyrimidine dimers and ~ 100 single-strand breaks in DNA due to ultraviolet radiation are registered per mean lethal dose in a genome of approximately 5×10^9 base pairs (see Elkind, 1980). Double-strand breaks in DNA are produced by ionizing radiation at about 50-100 per Gy, a 10- to 20-fold lesser frequency than the production of single-strand breaks (see Elkind, 1984). Although some kinds of lesions may be preferentially repaired in transcriptionally active, as opposed to inactive, regions of the genome, it is not likely that a cell can survive with a large amount of unrepaired DNA damage. As noted, both prokaryotic and eukaryotic cells may tolerate certain amounts of template damage (see Friedberg, 1985), but the frequencies at which such damage is incurred suggest that to survive a cell must repair a large amount of potentially genotoxic damage (Elkind et al., 1987). Therefore, it is likely that a low but significant frequency of inaccurate repair, i.e., misrepair resulting in a mutation, could occur in the course of a cell's recovery from a genotoxic treatment. The existence of such processes, called "SOS" repair in *E. coli*, have been demonstrated and shown to be mutagenic (Witkin, 1989). SOS-type repair has not been demonstrated convincingly in mammalian cells, however.

2.2.2.1 Repair in Mammalian Cells

An understanding of repair processes in mammalian cells is made difficult by their apparent complexity. In addition, it is uncertain how to make projections of mechanisms from bacteria to mammalian cells. For example, the bacterial genome does not contain chromatin--i.e., DNA-protein complexes consisting of histones and the structural proteins of the nuclear matrix--nor do bacterial genes consist of *introns* as well as *exons*. (Exons carry the genetic message of the gene.) Furthermore, the organization, replication, and segregation of the mammalian cell genome, which differs from that of the bacterial genome, is incompletely understood (Friedberg, 1985) and is known to involve complex relationships of nuclear and cytoplasmic structural proteins and enzymes (see Pienta, et al., 1989).

Much of our understanding of the processes of repair in human cells has come from studies using cells from people who have one of four repair-deficient, or repair-defective, human disorders; namely, xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Cockayne's Syndrome, and Fanconi's Anemia. The foregoing rare, recessively-inherited human disorders are associated with an increased risk of cancer (see Friedberg, 1985). In addition to these human-cell mutants, mouse- and hamster-cell repair-deficient mutants have been induced, isolated, and characterized (see Collins and Johnson, 1987; Cleaver, 1989).

The genetic complexity of these human and rodent repair-deficient mutants is made evident by the many complementation groups that are involved.

(Operationally, the existence of a complementation group is indicated if it can be demonstrated that the same phenotypic defect in two separate cells, such as a defect in repair, is not expressed when their genetic material is enabled to act in concert, for example, because the cells were fused.) In XP cells, for example, at least 15 genes are inferred to be involved in the repair of pyrimidine dimers that, in bacteriophage, can be repaired by a single gene (Walker, 1985). Although studies of these mutants have provided insights into the molecular steps in the repair of damage caused by ultraviolet radiation and certain chemicals, relatively little is known even in microorganisms about the mechanisms of the repair of lesions due to ionizing radiation such as double-strand breaks in DNA.

The identification and characterization of rodent-cell mutants that are hypersensitive to killing by ionizing radiation has, nonetheless, begun to shed light on the kinds of repair processes that respond to ionizing radiation (see Collins and Johnson, 1987; Cleaver, 1989). Repair of damage from ionizing radiation in prokaryotes and in yeasts is associated with excision and recombinational pathways. Because the latter are not understood in mammalian cells, their role in repair of x-ray type damage is ill defined (see Sankaranarayanan, 1982; Friedberg, 1985; Hanawalt et al., 1979). With AT cells, studies of the repair and misrepair of damage that may lead to chromosomal aberrations are illuminating some aspects of the defect in these cells and, as a result, suggestions have been proposed to explain why repair may proceed more effectively in normal cells (see Bridges and Harnden, 1982; Painter, 1982; Cornforth and Bedford, 1985).

Bloom's Syndrome is another human disorder that is of interest because cells from such individuals have been found to be deficient in ligating strands of DNA (Willis et al., 1987). Even though cells of the preceding type have not been identified as repair-defective, they have both an increased rate of spontaneous mutations, sister chromatid exchanges, and mitotic recombinations (Vijayalaxmi, et al., 1983).

It has been proposed that the association of DNA with protein in chromatin, or the protein matrix of the nucleus, can maintain broken fragments of DNA in proximal positions long enough to enable repair. In any event, the apparently high proportion of deletion mutations caused by ionizing radiation (see Thacker, 1986), compared to many chemical mutagens, indicates that genetic material is probably either lost or relocated in the genome during the repair of DNA breaks induced by radiation. From the foregoing, it would follow that a significant proportion of the repair events may involve errors. It is likely that the kinds of DNA-matrix associated enzymatic complexes, such as replisomes and structural elements of the nuclear protein matrix, are important in repair just as they are in the normal synthesis of DNA although this possibility has not been demonstrated as yet (see Pienta et al., 1989).

2.2.2.2 Repair, Mutation, and Cancer Initiation

It is evident that repair and mutagenesis and, therefore, repair and oncogenesis, are linked. The evidence indicates that the somatic mutations that may initiate tumorigenesis occur at specific, genomic loci in certain cells. An important example of such specificity associated with cancer is illustrated by Burkitt's lymphoma, a neoplasm for which the molecular biology has reached the point that enables the chromosomal locations associated with the disease to be

identified (see Nowell and Croce, 1986). (The molecular details of Burkitt's lymphoma are described in Subsection 2.3.2.2.)

As for other cancers, such as leukemias and solid tumors, thus far it appears that leukemias are associated with various specific chromosomal abnormalities (Rowley, 1984; LeBeau and Rowley, 1986). Many solid tumors also are likely to be associated with specific chromosomal rearrangements (see Brodeur et al., 1981; Yunis, 1983; Cavenee, 1989).

If a mutation causes a cell to become heterozygous for a so-called anti-oncogene gene (also referred to as a suppressor gene), then a homozygous recessive state could result, which could lead to neoplasia, if another mutation occurs at the same locus in the homologous chromosome, or if the remaining allele becomes nonfunctional in some other way like a chromosomal deletion or rearrangement. This sequence of genetic changes has been demonstrated for the cancer of the eye, retinoblastoma (e.g., Cavenee et al., 1983). Ionizing radiation is known to be able to cause the kinds of mutations and chromosomal changes that could result in the homozygous state identified with retinoblastoma.

2.3 UNCONTROLLED CELLULAR GROWTH, MOLECULAR MECHANISMS

Cancer is a disease of uncontrolled growth and this property unifies all neoplasms. The cellular mechanisms, which are involved in the major multiple steps of *initiation*, *promotion*, and *progression*, may differ somewhat from one etiological agent to another, and probably among neoplasms at different sites and of different histological appearance. Still, a unifying feature of all cancers is the lack of regulated growth of the cells that characterize the tumor. Examples of the cellular/molecular changes, which can give rise to unregulated growth, are discussed in this Section.

Although the major steps in neoplasia are generally classified as above, at some sites and for some types of tumors additional steps have been identified. In addition, at a cellular level indicators of changes in properties, which are believed to relate to neoplastic growth, have been identified; these steps are often referred to as *initiation*, *immortalization*, *clonal expansion*, and *malignant progression*, and are believed to overlap the steps that occur *in vivo*. Experimentally, other changes in cellular properties have been observed but these are considered to be further refinements, or substeps, of the major steps noted above. For example, a reduction in the dependence on serum for growth *in vitro* has been taken as one corollary of the loss of regulation of growth; the appearance of aneuploidy has been associated with immortalization *in vitro* [as well as with progression *in vivo* (Hanahan, 1988)]; and the loss of anchorage-dependence for growth has been associated with a change connected with progression.

It is apparent from the preceding that imprecision exists in the various terms and concepts that are applied to the cellular/tissue changes that culminate in a neoplasia. This fuzziness reflects the incomplete knowledge of the causes of neoplastic growth and how such growth develops, as well as the recognition that cancer is a relatively nonspecific name, encompassing a variety of conditions involving unregulated growth among which significant differences exist, just as the term "infection" is applied to a large group of diseases.

2.3.1 Proto-Oncogenes [or p-Oncogenes]

The tumorigenic and molecular properties of retroviruses have served to identify a class of genes in mammalian cells that have been shown to play a critical role in oncogenesis and, therefore, in the neoplastic transformation of cells. Evidence of the past decade indicates that the constitutive forms of these genes have roles to play in normal cell growth and differentiation (Fisher et al., 1989) whereas their inappropriate expression or abnormal counterparts may produce neoplastic transformation.

In normal cells, these genes are called *proto-oncogenes*, *p-oncogenes*, or frequently just *oncogenes* (Knudson, 1985; Pearson and Rowley, 1985; Buckley, 1988; Haluska et al., 1987; Land et al., 1983; Fisher et al., 1989). As a group, these genes have DNA sequences, and code for functions, that have been well conserved throughout evolution. Table 2.1 lists 17 examples of some of the 50 human oncogenes that are known at the present. Also tabulated are their chromosomal locations, and these oncogenes derived and/or functions. Genes analogous to those in Table 2.1 are found in the genomes of other mammalian species as well as in other eukaryotes, such as in yeasts and insects, and even in plants.

In addition to those in Table 2.1, new oncogenes are being identified as the cellular and molecular properties of different tumors become more fully understood. The information content of some oncogenes--i.e., the code contained in their exons--has been shown to be transducible into cells by viruses. But there are a number of other genes, which play a role in normal growth, division, cell-to-cell communication, and differentiation, that can constitute oncogenes because in principle they can contribute to unregulated growth and/or differentiation if they are mutated, functionally lost, or expressed inappropriately.

Considerable evidence from human and animal material indicates that many different kinds of genetic changes, resulting in the activation or abnormal expression of oncogenes, are associated with cellular transformation and neoplasia. These changes and their effects are discussed below. Evidence also has been developed indicating that the collaborative processes of at least two oncogenes in some instances may be involved in neoplastic growth (Land et al., 1986; see Weinberg, 1989).

2.3.2 Genetic Changes

The products of p-oncogenes, their functions, and consequently the normal roles of these genes are not entirely understood. Still, it is believed that ionizing radiation, and other physical and chemical inducers of neoplasia in general, affect p-oncogenes or their expression via one or more of the following mechanisms.

2.3.2.1 Point Mutation

One or a few base-pairs may be changed resulting in an altered gene product that leads to, or contributes to, the loss of the regulation of growth (see Tabin et al., 1982; Barbacid, 1986, 1987). Examples of point mutations in the *ras* oncogene are shown in Table 2.2 (see Levinson, 1986).

TABLE 2.1

<u>Oncogene Symbol</u>	<u>Chromosomal Location</u>	<u>Oncogene Name and/or Function</u>
<i>c-myb</i>	6q22-q23	avian myelo-blastosis viral (v- <i>myb</i>) oncogene homolog; nuclear function
<i>c-myc</i>	8q24	avian myelo-blastosis viral (v- <i>myc</i>) oncogene homolog; nuclear function
<i>c-fos</i>	14q24.3-q31	murine FBJ osteosarcoma viral (v- <i>fos</i>) oncogene homolog; codes for a protein that enhances in AP-1 transcription factor
<i>c-jun</i>	1	member of AP-1 family of transcription factors
<i>c-sis</i>	22q12.3-q13.1	platelet derived growth factor beta polypeptide; simian sarcoma viral (v- <i>sis</i>) oncogene homolog
<i>c-erbB</i>	7p13-p12	epidermal growth factor receptor, EGFR, formerly avian erythroblastic leukemia viral (v- <i>erb-b</i>) oncogene homolog
<i>c-fms</i>	5q33-q34	colony stimulating factor 1 receptor
<i>c-erbA1</i>	17q11-q21	erythroblastic leukemia viral (v- <i>erb-a</i>) oncogene homolog; thyroid hormone receptor
<i>c-erbA2</i>	3 or 17q21	avian erythroblastic leukemia viral (v- <i>erb-a</i>) oncogene homolog 2
<i>c-src</i>	20q12-q13	avian sarcoma viral (v- <i>src</i>) oncogene homolog; tyrosine-specific protein kinase
<i>c-abl</i>	9q34	Abelson murine leukemia viral (v- <i>abl</i>) oncogene homolog; tyrosine-specific protein kinase
<i>c-Ha-ras1</i>	11pter-p15.5	Harvey rat sarcoma 1 viral (v- <i>Ha-ras1</i>) oncogene homolog Guanine nucleotide binding protein with GTPase activity
<i>c-Ki-ras1</i>	6p12-p11	Kirsten rat sarcoma 1 viral (v- <i>Ki-ras1</i>) oncogene homolog Guanine nucleotide binding protein with GTPase activity
<i>c-Ki-ras2</i>	12p12.1	Kirsten sarcoma 2 viral (v- <i>Ki-ras2</i>) oncogene homolog
<i>c-fes</i>	15q25-q26	feline sarcoma viral (v- <i>fes</i>) oncogene homolog; tyrosine-specific protein kinase
<i>c-fgr</i>	1p36.2-p36.1	Gardner-Rasheed feline sarcoma viral (v- <i>fgr</i>) oncogene homolog; formerly c- <i>src2</i> ; tyrosine specific protein kinase
<i>c-mos</i>	8q11 or 8q22	Maloney murine sarcoma viral (v- <i>mos</i>) oncogene homolog; serine/threonine protein kinase

TABLE 2.2
MUTATIONS IN THE RAS ONCOGENE

Oncogene	Tumor Cell Line	Altered Codon	Nucleotide Exchanged	Amino Acid Exchanged
Ha-ras1	Bladder carcinoma EJ/T24/J82	12	G → T	gly → val
Ha-ras1	Spontaneous	12	G → A	gly → asp
Ha-ras1	Lung carcinoma	61	A → T	glu → leu
Ki-ras2	Lung carcinoma	12	G → T	gly → cys
Ki-ras2	Colon carcinoma	12	G → T	gly → val
N-ras	Neuroblastoma	61	C → A	glu → lys

2.3.2.2 Chromosome Rearrangement

As mentioned in Subsection 2.2.2.2, a normal p-oncogene may be translocated into a transcriptionally active region of the same chromosome or some other chromosome such as the region in a chromosome of a B-type lymphocyte that contains the genetic information for immunoglobulin production as in Burkitt's lymphoma (see Nowell and Croce, 1986). In Burkitt's lymphoma, ~75 percent of the tumors have been found to involve an 8:14 reciprocal translocation. In the remaining cases, either an 8:22 (~16%) or an 8:2 (~9%) translocation is involved (Nowell and Croce, 1986). It appears that in all instances of this disease, the *c-myc* oncogene is brought into juxtaposition with genes coding for one or another portion of the immunoglobulin molecule; see Figs. 2.2 and 2.3, and Table 2.1 relative to the details of these translocations and the specification of chromosomal locations. In all cases the break points of the translocations occur on chromosome 8 immediately adjacent to the *c-myc* oncogene, Fig. 2.2. Where chromosome 14 is involved, the acentric or terminal portion of the fragment of chromosome 8 containing *c-myc*, which is immediately adjacent to the break point, is joined to chromosome 14 at its break point and immediately adjacent to the genes in the latter chromosome that code for the constant region of the antibody heavy chain, IgG. In the remaining cases, the *c-myc* gene is retained on chromosome 8, but genes that code for the light chains of the antibody, which are on chromosomes 2 and 22, are translocated to chromosome 8.

Of the some 50 known human oncogenes listed in Table 2.1, 17 are located in Fig. 2.3. Figure 2.3 is an ideogram, a reference map, of the 22 autosomes plus 2 sex chromosomes in which the genome of a human cell is contained. (Most human somatic cells are diploid and contain, therefore, 44 autosomes as well as 2 sex chromosomes.) The translocation of the *c-myc* gene to the region of one of the active promoters associated with the transcription of the immunoglobulin genes raises the likelihood that Burkitt's lymphoma is a consequence, at least in part, of the overexpression of the *c-myc* oncogene and consequently in the increased activity of its protein product (Croce, 1986).

Further examples of neoplasms associated with chromosomal abnormalities, including translocations and deletions, and particularly those associated with the loss of tumor-suppressor genes, are listed in Table 2.3 and discussed below.

2.3.2.3 Gene Amplification

The copy number of a p-oncogene may be increased by as much as an order of magnitude or even more in some instances of gene amplification. As a result, an otherwise normal gene product may be overproduced, a change that may lead to a loss of growth regulation (see Bishop, 1983a,b). Table 2.3 lists examples of oncogenes and the human tumors with which they have been associated.

2.3.2.4 Viral Induction

Control elements, such as promoters of viral DNA, may activate neoplastic potential by becoming inserted into a position in a chromosome from which they can alter the expression of a normal p-oncogene. Additionally, when the DNA derived from the RNA of a retrovirus is inserted into the genome of a mammalian cell (the genome of a retrovirus consists of RNA that can be transcribed into its complementary DNA), in the process normal oncogenes may become mutated leading to the altered expression of that gene. An additional role for a retrovirus in cell transformation is potentially via transduction. That is, the RNA

Chromosomal Translocations in Burkitt's Lymphoma

Fig. 2.2 Chromosomal translocations that are observed in Burkitt's lymphoma. V_H and C_H are the genes encoding immunoglobulin heavy chain variable and constant chain regions, respectively. V_K , V_λ and C_K , C_λ are the genes encoding immunoglobulin light chain variable lambda and kappa regions, and constant chain lambda and kappa regions, respectively. The translocation that occurs in ~75% of cases is abbreviated t(8;14) (q24;q11) to indicate that a translocation has occurred between chromosome 8 and 14 with the exchange points in the long (q) arm of chromosome 8 at position q24 and the long (q) arm of chromosome 14 at position q11. The same notation applies for the other translocations. The map, or ideogram, of a set of human chromosomes is shown in Fig. 2.3.

Fig. 2.3 Ideogram of chromosomes of a human cell. Map locations are given by designating in the order: 1) the chromosome number; 2) the chromosome arm, p (short arm) or q (long arm); 3) a number designating the region in the particular arm; and 4) the particular band in that region. For example, the arrow to the right of chromosome 1 points to the map location 1p34; that is, chromosome 1, short arm p, region 3, and band 4 (G-light band). Various technique are used that reproducibly produce bands which can be identified in the light microscope.

TABLE 2.3

Examples of Some Chromosomal Deletions and Translocations Associated with Various Cancers, and Likely or Possible Genes Involved¹

Chromosome Abnormalities ²	Type of Cancer	Likely or Possible Gene Involved ³
del(1)(p36-p32)	neuroblastoma	c-fgr, B-lym
del(3)(p14-p23)	small cell lung cancer	(tumor suppressor)
del(5)(q12-q35)	acute non-lymphocytic (myeloid) leukemia; (secondary leukemias ⁴)	c-fms
del(5)(q)	colorectal carcinoma	(tumor suppressor)
del(11)(p13)	Wilms tumor	(tumor suppressor)
del(13)(q13.3-q14.3)	retinoblastoma	(tumor suppressor)
del(13)(q14)	small cell lung cancer	(tumor suppressor)
del(13)(q14)	breast cancer	(tumor suppressor)
t(9;22)(q34;q11)	chronic myelogenous leukemia	c-abl
t(8;14)(q24;q11)	Burkitt's lymphoma ⁵ (acute lymphoblastic leukemia sub-class 3)	c-myc
t(8;22)(q24;q11)	Burkitt's lymphoma ⁵	c-myc
t(2;8)(p12;q24)	Burkitt's lymphoma ⁵	c-myc
t(6;14)(q21;q24)	ovarian carcinoma	c-myb
t(11;22)(q24;q12)	Ewing's sarcoma	PDGFB

¹For this table a few selected examples were taken from Human Gene Mapping 2, Cytogenetics and Cell Genetics 46, (1987) and from Stanbridge, 1988. In addition to those listed, deletions or translocations have been associated with nearly every solid tumor and leukemia studied to date.

²The notation del(1)(p36-p32) refers to a deletion in chromosome (1) somewhere in the short arm, p, between the regions p36 and p32. A similar notation applies for other deletions. The notation t(9;22)(q34;q11) refers to a translocation between chromosomes 9 and 22 with the exchange points being at position q34 in the short(q)arm of chromosome 9 and position q11 in the short(q)arm of chromosome 22. A similar notation applies for other translocations. The complete human chromosome map is shown in figure 2.3.

³See also Table 2.1 and figure 2.3.

⁴Monosomy for the entire chromosome 5 is common for such tumors.

⁵See also figure 2.2.

transcribed from a mutated p-oncogene may become incorporated into the RNA of a retroviral genome. If the DNA transcribed from the new RNA genome becomes integrated into the genome of another cell, the recipient then may become transformed. Alternatively, after or during the process of the transduction of a normal oncogene, it may become mutated. In either case, the recipient cell becomes transformed as a result of, or in connection with, the expression of the mutated p-oncogene that it has incorporated.

2.3.2.5 Altered Regulation

A change may occur in the *cis* or *trans* control element(s), promoters, enhancers, or transcription factors of a p-oncogene thus significantly altering, either up or down, its rate of transcription. This change could lead to the over- or underproduction of a gene product which could affect the control of growth via an imbalance in the factors affected by the product of the oncogene.

2.3.2.6 Functional Loss of Anti-Oncogenes [or Tumor-Suppressor Genes]

The idea that tumor formation could be associated with a genetically recessive trait came originally from the demonstration that tumorigenesis was suppressed in certain hybrid cells formed by the fusion of a normal and a malignant cell (Harris et al., 1969). More recent work has indicated that if both alleles of certain types of tumor-related genes are lost or inactivated, tumors may result (see Cavanee et al., 1983; Knudson, 1985; Benedict et al., 1987; Stanbridge, 1988). These observations suggest that the susceptibility to cancer is hereditary. They suggest also that the loss of an anti-oncogene, or tumor suppressor gene, whose products normally regulate differentiation, can result in a loss of growth control (Klein and Klein, 1985; Klein, 1987). The loss of function of an anti-oncogene could result from a point mutation, from a recombinational event that causes the anti-oncogene to become unexpressed, or to the loss of the entire chromosome that carries the gene, as in nondisjunction. (Nondisjunction is an uneven separation of chromosomes during mitosis resulting in a distribution of one and three copies of a given autosome in the two daughter cells). Table 2.3 includes a listing of chromosomal deletions associated with a number of human anti-oncogenes and the neoplasms associated with them.

2.3.2.7 Protein Regulation of Gene Transcription

The phenotypic response of a cell to its environment may be governed by the regulation of gene expression via extracellular signals. Studies with prokaryotes have shown that proteins capable of regulating transcription can bind to specific regions of DNA. A fundamental question has been how the specific binding is accomplished given the quite uniform structure of double-helical DNA. The answer that appears to be emerging is that in both prokaryotic and eukaryotic cells regulatory proteins detect and bind tightly to specific sequences of base pairs. Four classes of such DNA-binding proteins have been identified: a) those with the "helix-turn-helix" motif (the so-called homeobox proteins); b) those with the "zinc finger" motif; c) those with the "helix-loop-helix" motif; and d) those with the "leucine zipper" motif (see Johnson and McKnight, 1989).

Recently, it has been shown that several of these regulatory proteins have amino acid sequences related to the products of the *myc*, *fos*, and *jun* proto-oncogenes and, in turn, that these products can regulate the expression of specific genes. Thus, mutations in the genes for these regulatory proteins can regulate upward or downward specific genes including the genes that code for the

regulatory proteins themselves. Mutations of this kind caused by ionizing radiation, or other agents, could lead to unregulated cell growth and hence to neoplasia.

2.3.2.8 Altered Processing and Transport of RNA

The over- or underexpression of genetic information can be influenced by quantitative changes, in the transfer of genetic information from the site of transcription in the nucleus to the site of translation in the cytoplasm, as well as by qualitative changes in the information itself. For example, pre-mRNA (unprocessed messenger RNA) of the normal cellular gene *c-myc* has been shown to be transcribed at a high rate in nontransformed cells that were reversibly arrested in the G₀/G₁-phase of the cell cycle (i.e., at the beginning of the phase of the cycle preceding DNA synthesis; Blanchard et al., 1985). Mature *c-myc* mRNA was not formed until the arrested cells were stimulated by serum to enter the G₁-phase, implying a posttranscriptional modification of the genetic information (Blanchard et al., 1985; see also Cole, 1985). Certain mutations have been shown to lead to the production of mRNA's that have an increased ability to be translated into active protein (Cole, 1986). Also, it has been shown that the enhanced transport of mRNA sequences from the nucleus to the cytoplasm can occur when the promoter of the gene in question has been induced to act in *trans* (i.e., downstream from the gene or from a location on a chromosome different from the one on which the gene is located; de la Pena and Zasloff, 1987).

2.4 PHENOTYPIC EXPRESSION OF ONCOGENE ACTIVITY

The gene products associated with specific p-oncogenes have different properties and different sites of action. In general, with respect to the cell of origin, they may function on or in the cell's outer membrane, in the cytoplasm, or in the nucleus. illustrated in Fig. 2.4. Examples of several constitutive, i.e., normal, p-oncogenes and their products are shown in Fig. 2.4; a further listing is in Table 2.1. The sites of action and the products on a number of p-oncogenes follow.

2.4.1 Oncogenes for External & Cell-Surface Factors

- c-sis*: platelet-derived growth factor, PDGF
- c-erbB*: receptor for epithelial growth factor, EGF β
- c-fms*: receptor, colony-stimulating factor one, CSF-1
- c-erbA*: receptor for thyroid hormone.

These gene products act at the plasma, external envelope of the cell, see Fig. 2.4 for examples.

2.4.2 Oncogenes for Signal Transducers, Protein kinases, and Nucleotidases

- c-src*, *c-abl*, *c-Ha-ras*, *c-Ki-ras* (in the plasma membrane)
- c-fes*, *c-fgr*, *c-mos* (in the cytoplasm)

The action of the products of these oncogenes is mainly confined to the plasma envelope and the cytoplasm of a cell, see Fig. 2.4 for examples.

Fig.2.4 A simplified illustration of the sites of action of several proto-oncogenes. Oncogene products are in bold type; the growth factors that interact with some of the receptors coded by oncogenes are shown. The products of oncogenes are known to act at the outer membrane, in the cytoplasm, and in the nucleus of a cell.

2.4.3 Oncogenes for DNA and Nuclear Matrix-Binding Protein

c-myc, *c-jun*, *c-fos*, and *c-myb*

The action of the products of these oncogenes is confined to the nucleus, see Fig. 2.4 for examples.

2.5 DOMINANT ONCOGENES VS. ANTI-ONCOGENES/TUMOR-SUPPRESSOR GENES

With respect to tumorigenesis in general, two quite different modes of action have been identified of how oncogenes effect the transformed phenotype and neoplastic growth.

2.5.1 Dominant Oncogenes

The modifications of p-oncogenes, which give rise to neoplastic transformation, are generally found to function in the heterozygous state and therefore they are genetically dominant. Dominant transforming genes were discovered through identification of viruses that were able to induce tumors in infected animals, and/or to transform morphologically cultured animal cells. The isolation of oncogenes, with tumorigenic or transforming activity, from both DNA- and RNA-containing viruses, and the discovery that certain retroviruses had incorporated cellular genes, demonstrated that dominantly-acting genes were involved in virus-induced tumorigenesis (Varmus, 1984; Bishop, 1983a).

The data in Table 2.2 illustrate the effect of genes that act dominantly, and were obtained in the following way. DNA, extracted from tumors and introduced into NIH 3T3 mouse fibroblasts that were otherwise nontumorigenic, was found to be able to transform these cells. As a result of the transfection, the 3T3 cells produced tumors at the site of their inoculation into immune-suppressed mice. Analysis of the *ras* gene in the DNA extracted from the tumors demonstrated that this gene contained a single base-pair change at either the 12th or the 61st codon. These coding changes would have given rise to the amino acid substitutions that are indicated in Table 2.2 (see Levinson, 1986).

In the foregoing, activation of the *ras* gene clearly resulted from mutations, i.e., base changes. Activated oncogenes have not been thought to be involved in the genetic predisposition to cancer, however, because the abnormal expression of oncogenes in the developing embryo has been considered to be incompatible with the survival of the fetus. Furthermore, mutations in the oncogenes of human germ-line cells (see Fig. 2.1) thus far have not been reported.

The role of oncogenes in the multistep development of neoplasms is under study via the use of transgenic mice. These mice are generated by microinjecting, into a fertilized mouse egg, DNA containing the gene of interest. The egg is then introduced into the oviduct of a female mouse giving rise, at a reasonable frequency, to an adult animal whose somatic and germinal tissues contain the foreign DNA of interest integrated into the genomes of the cells (Brinster et al., 1984; Hanahan, 1988, 1989).

It has been shown with transgenic mice that a specific oncogene can give rise to specific, heritable tumors. For example, a *c-myc* oncogene, under the control of a promoter of a mouse mammary tumor virus to insure its expression,

produced clonal mammary carcinomas in transgenic mice (Stewart et al., 1984; Adams et al., 1985). Although a promoter-linked c-myc gene--i.e., a c-myc gene whose transcription was assured--may have been present in all of the cells of the animal, only mammary tumors occurred suggesting that the c-myc gene was a necessary condition for mammary tumor formation, but not a sufficient requirement for tumor formation in general.

In other studies with transgenic mice, several kinds of tumors were induced--including those of the lung, salivary gland, bone, and pancreas--when both c-Ha-ras and c-fos were transfected. But in all instances where a single oncogene was used in these studies, only a preneoplastic state, characterized by hyperplasia, was observed. Apparently, the changes that result from both genes were required to bring about a frank cancer suggesting the collaboration of the products of two genes in these instances (Hanahan, 1988).

Additional support for the idea that a single oncogene is insufficient for neoplastic transformation comes from experiments with normal rat embryo fibroblasts in culture. The introduction of a single oncogene, a mutated ras gene, into these cells did not produce neoplastic transformation, whereas the simultaneous introduction of both the ras and myc oncogenes did (Land et al., 1983; 1986). Results such as these imply that a single oncogene may not be sufficiently dominant, in all instances, to overcome the genes that maintain normalcy, suggesting that a mutation in a p-oncogene may be only a predisposing event in germ-line cells. These observations also support the inference that there are genes whose products place a check on unlimited growth.

2.5.2 Anti-Oncogenes/Tumor-Suppressor Genes

Genes, called anti-oncogenes or tumor-suppressor genes, were first made evident in human studies of familial cancer from which it was concluded that tumor formation requires two apparently independent events; that is, a secondary somatic mutation in addition to an initial, inherited genetic change (Cavenee et al., 1983; Knudson, 1971, 1978, 1985; Koufos et al., 1984; Fearon et al., 1985; Pearson and Rowley, 1985; Lundberg et al., 1987; Stanbridge, 1988). The two independent events were imagined to be mutations, or losses of both copies of a gene that was recessive for oncogenesis, but dominant for normalcy. For example, the first event could be a mutation or a deletion in a germ cell; a change that is recessive because, from this alone, the offspring does not develop a neoplasm. In the somatic cell of the offspring that does develop cancer, the second event could be a point mutation, a deletion, a loss of the activity of the normal gene as a result of DNA recombination, or a loss because of chromosomal nondisjunction at mitosis. [In some instances, more than one second event has been found to be associated with the same neoplasm (see Sager, 1989).] Mechanisms such as these have been found in retinoblastoma (Knudson, 1985), and are likely to apply in reference to other neoplasms (Pathak et al., 1982; Whang-Peng et al., 1982; Koufos et al., 1984; Seizinger et al., 1986; Solomon et al., 1987). Figure 2.5 shows diagrammatically the action of an anti-oncogene. Additional suppressor genes are listed in Table 2.3.

Hypotheses have been proposed that bring together the notions of dominantly-acting oncogenes, and recessively-acting anti-oncogenes. For example, an oncogene may provide a product that normally regulates the expression of a second oncogene, one that could act dominantly if it were not controlled. Thus, the first gene acts like an anti-oncogene. A cancer process could be initiated

Fig. 2.5 An anti-oncogene/suppressor gene representation of the induction of neoplastic growth.

Panel 1 A normal cell containing two allelic anti-oncogenes (solid bands on the upper chromosomes), which code for proteins that maintain the cell in its normal, non-transformed state. The proteins of these genes may act by inhibiting the action of oncogenes (stippled bands on the lower chromosomes).

Panel 2 A cell that has lost one allele of the pair of anti-oncogenes, either as an inherited mutation or as the result of a post-zygotic mutation (see Fig. 2.1), is still able to suppress a neoplastic transformation because the anti-oncogene acts dominantly to maintain normalcy. However, individuals with this inherited loss of homozygosity--that is, who are genetically +/- (Fig. 2.1)--are at a greatly increased risk of contracting cancer.

Panel 3 An initiated, transformed cell results when the second allele of the anti-oncogene pair is inactivated in some way. Changes known to cause inactivation include point mutations, methylation-mediated DNA modifications, and large-scale chromosomal rearrangements such as mitotic exchanges, interstitial and terminal deletions, and nondisjunction

either because the second oncogene suffers a change in its regulatory region that frees it from the control of the product of the first, or because the product of the first oncogene becomes ineffective in controlling the action of the second (Knudson, 1988). In either case, the final result could be the abnormal expression of an oncogene. Also, the process could be initiated by a heritable change resulting from unrepaired or misrepaired genetic damage due to radiation or another causative agent.

2.6 GROWTH FACTORS AND CELL-CELL COMMUNICATION

The effectors of abnormal growth may be factors that stimulate, or that fail to control, proliferation that is otherwise normal.

2.6.1 Growth Factors

Extracellular factors, which influence the growth of cells via cell-to-cell signalling, are of three types (see Fig. 2.6): *endocrine*, in which chemical signals like hormones are transmitted to a cell via the blood stream; *paracrine*, in which a cell secretes a signal that influences the growth of an adjacent cell via receptors on the plasma membrane of the latter cell; and *autocrine*, in which the secretory/target cell relationship exists in one and the same cell. Oncogene products are known that are involved in all three mechanisms (Marks, 1987; Sinkovics, 1988).

2.6.2 Cell-Cell Communication

In addition to growth control via extracellular signalling, it has been demonstrated that control can also be effected between cells through transmembrane, cell-to-cell communication. To affect tissue organization, as well as to direct cell communication, several types of interconnections exist between cells. These include: *tight junctions*, to prevent leakage past adjacent cells; *desmosomes*, to anchor adjacent cells together or to a basement membrane; and *gap junctions*, to allow cells to pass small molecules between them including mono- and divalent ions.

Gap junctions consist of small pores, about 2 nm in diameter, that interconnect the cytoplasm of two adjacent cells. Six protein subunits, arranged around an axis transverse to each of the plasma membranes that are joined, lock together to form the connection between cells. These proteins react to the intracellular concentration of Ca^{2+} ions, cyclic adenosine monophosphate, and other molecules by changing their orientation, and thus the size of their opening, in order to control the passage of molecules between cells. The size of the pores limits the passage of molecules to those with molecular weights of less than ~2,000 daltons.

The importance of gap-junctional communication between cells stems from the evidence that transformed cells communicate poorly with nontransformed cells (e.g., Mehta et al. 1986) as well as with each other. As a result, it is believed that shifts in the regulation of growth could result from an imbalance in small, intracellular molecules resulting in abnormal concentrations in the cell destined to become transformed. Another mechanism, for which data also exist, is that pivotal differences between normal and transformed cells can result from changes in the effective pore size of the gap junctions. This idea is connected with the prompt effects of tumor promoters, like the phorbol esters,

Fig. 2.6 Examples of the modes of action of growth factors (69).

in reducing the communication between cells and thus promoting the proliferation of an initiated cell (Yamasaki and Fitzgerald, 1988; El-Fouly et al., 1987). An association has been made between the latter idea and the transforming properties of a retrovirus, the Rous sarcoma virus v-src. Rose, Lowenstein, and their collaborators have proposed that the particular tyrosine kinase activity, which is encoded by the v-src oncogene, correlates with the reduced junctional permeability and increased cell growth of transformed cells (Atkinson et al., 1981; Azarnia et al., 1988). The enzymatic phosphorylating activity of the src-encoded kinase differs from that of the kinase produced by the normal src gene in nonvirally infected cells.

Thus, specific proteins and other molecules, which may influence communication, may be regulated to increase or decrease their activity as a consequence of changes produced in one or more oncogenes. In addition, at critical stages in the multistep process of tumorigenesis the societal relationships among the cells in question may be important. These relationships, in turn, are governed by particular gene products that are themselves liable to alteration due to a mutation by ionizing radiation or other agents.

2.7 PROMOTION

As already indicated, oncogenesis proceeds through a series of steps during which cells progressively acquire the phenotypic characteristics associated with neoplasia. During the process of promotion, cells that are already initiated are acted upon to increase the probability of their giving rise to a recognizable neoplasm. The process of promotion involves traversing one or more steps that can lead to neoplasia and malignancy. Promotion may involve the induction of new cellular phenotypes, as well as the selection of a subpopulation among the initiated cells that already may be present (Colburn et al., 1987). Figure 2.7, after Nowell (1976), is a scheme of how an initiated cell might give rise to a primary tumor as well as to metastases.

2.7.1 Clonal Expansion

A major effect of promoters is to elicit the specific expansion of initiated cells, that is, clonal expansion. While cell proliferation may be considered a major, or even essential, step in promotion, there may be more than one mechanism by which it can occur. In general, hyperplastic conditions are often precursors of neoplasia (Colburn et al., 1987; Hanahan, 1988). Studies using transgenic mice have demonstrated the importance of cell proliferation in oncogenesis Hanahan (1988, 1989). As discussed in Section 2.5, the establishment of stable lineages of transgenic mice, with hereditary predispositions to specific cancers, strongly supports the likelihood that oncogenes are causally related to neoplasia. A common feature in these studies has been the development of hyperplastic conditions. Although specific oncogenes were used to induce unregulated cell proliferation, when used singly they were unable to induce tumors apparently because additional changes beyond oncogene-induced hyperplasia were required.

All neoplasms do not necessarily arise from hyperplastic conditions. Foci of dysplasia are also often sites of neoplasm formation. In dysplasia, affected cells may take on many aberrant histological characteristics some of which are often associated with neoplastic cells. Dysplastic cells are considered preneoplastic because cancers frequently arise from them. Even in these

Fig. 2.7 Theoretical pathways for the development of neoplasia through the steps identified as *initiation*, *promotion*, and *progression*. **S** and **G** represent somatic and germ cells, respectively. For a somatic cell, **1** could be the initiating genotoxic damage caused by an agent like radiation or a chemical carcinogen. For a germ cell, **1** could be an inherited recessive lesion as in retinoblastoma. Either of these changes could lead to a cell in growth state **P**, which is predisposed to the development of neoplasia, and could have increased proliferative potential. Step **2** could be a secondary alteration of the genome conferring neoplastic potential to form either a benign tumor cell, **B**, or a malignant tumor cell, **M**. These changes could result from the loss of certain methyl groups in the genome leading to a benign growth, or to the loss of heterozygosity for an anti-oncogene leading to a malignant neoplasm. Step **2** could include the clonal expansion of an initiated cell and would be identified as promotion. A further change in **B** represented by step **3** could give rise to either a clone of another variant of a benign neoplasm, **B'**, or to a malignant growth, **M'**. Step **4** represents a further genetic change in **M** to **M'** representing the genetic instability that exists in many types in many types of malignant cells. Nonviable cells **N** may also be produced as a result of genetic instability. A further step(s) in the progression of malignancy is represented by **5**, a genetic change that may trigger the conversion of a cell in malignant clone **M** to one with metastatic potential, **MT**, and other properties of progression.

instances, there may be some hyperplastic growth of aberrant cells although the amount is usually small.

2.7.2 Mechanisms of Promotion

Mechanisms of the stimulation of division by promoters may be direct or indirect. Cell proliferation can be general, such as the compensatory repopulation of an injured tissue, or more specific, such as a direct growth stimulation of initiated cells by hormones or growth factors. The selective elimination of normal cells has also been observed. For example, the action of a promoter of the phorbol ester type can be mitogenic in preneoplastic and neoplastic cells in colonic epithelium but not in the normal epithelium (Colburn et al., 1987). Those cells that respond to the promoter are induced to secrete a compound, plasminogen activator, that selectively destroys the less committed cells in the mixed population. As an example of a promoter inducing a new cellular phenotype, Krieg et al. (1988) demonstrated that a phorbol ester could induce the overexpression of a number of oncogenes in neoplasms of mouse skin which were not overexpressed in normal or even in hyperplastic skin.

Mice can be bred for susceptibility to skin carcinogenesis, a process that also selects for sensitivity to promoters. Genes have been identified that control sensitivity to promotion. Thus, the genetic loci for initiation and promotion are separable (Colburn et al., 1987).

Ionizing radiation is generally thought to be a more effective initiator than a promoter. However, at doses high enough to induce significant tissue injury (i.e., cell killing), compensatory repopulation by itself can serve as a promotional stimulant presumably because the division process may serve clonally to expand the population from an initiated cell.

2.8 PROGRESSION

As noted, the development of a malignant neoplasm may involve a stepwise process (see Fig. 2.6). As a clone of cells develops from an initiated progenitor, new cell populations may be generated frequently with increasing probabilities of more malignant behavior at each subsequent step. This process constitutes *progression* as discussed below.

2.8.1 Cellular Heterogeneity and Clonal Evolution of Neoplasms

Frequently, primary neoplasms consist of multiple populations of neoplastic cells with heterogeneity in their degree of malignancy. This heterogeneity covers a wide range of biochemical, immunological, and biological characteristics including diversity of chromosomal composition, cell-surface receptors, intracellular and excreted enzymes, morphologies, growth properties, sensitivity to chemotherapeutic drugs and to radiation, and ability to invade normal tissues and thus to metastasize to other sites in the body (Fidler, 1988). The phenotypic characteristics most often associated with malignancy include autonomous growth, invasion, and metastasis (Farber, 1984). Development of cellular heterogeneity is a prime manifestation of neoplastic progression (Kerbel et al., 1988).

As the foregoing suggests, the cells comprising a neoplasm may change rapidly with the emergence of new subpopulations. Even though a neoplasm may

have a clonal origin, promotion followed by progression can lead to non-uniformity of cell type by the time a frank neoplasm is detected. Neoplasms of 1 cm³ contain about 1×10^9 cells, which would have required some 30 cell doublings, indicating that even relatively small, clinically recognizable neoplasms are well advanced in their natural history. Furthermore, at a later stage, neoplasms can once again become characterized by the presence of a single, dominant clone (Kerbel et al., 1988). For the latter situation to occur, a highly malignant cell may appear, which has enough growth advantage to overgrow earlier-appearing variants.

2.8.2 Invasion

Progression is frequently accompanied by invasion and metastasis. Invasion is the movement of neoplastic cells away from the site of the primary neoplasm, although they are still confined to a localized region in the tissue or organ of origin. Characteristics associated with neoplastic cells are probably important in the process of invasion, such as loss of contact inhibition, increased motility, and the production of digestive, extracellular enzymes (Wheelock and Robinson, 1983).

In reference to digestive enzymes, interactions between neoplastic cells and the extracellular matrix may be particularly important. Liotta (1984) proposed a three-step mechanism of invasion. In Step 1, the attachment of a neoplastic cell may be mediated through cell surface receptors for glycoproteins, like laminin and fibronectin. In Step 2, the neoplastic cell secretes digestive enzymes capable of lysing the proximal extracellular matrix and the basement membrane. And in Step 3, the cell breaks away from its immediate neighbors and migrates into the region of lysis.

Type IV collagen is a major protein contained in basement membranes. Enzymes that can digest basement membranes, such as Type IV collagenase, have been shown to be associated with invasive ability (Liotta, 1984). For example, in mouse skin the oncogene that codes for the protease transin appears to play a role in the progression of benign papillomas to invasive carcinomas (Matrisian et al., 1986). Laminin, which is a protein and a component of basement membranes, is important in cell attachment, migration, and growth. It has been shown that laminin receptors may be altered in carcinoma cells, and that neoplastic cells with increased ability to attach to laminin, have a much increased metastatic potential (Liotta, 1984).

2.8.3 Metastasis

Local invasion into the vasculature is important in the initial stages of metastasis. Thereafter, tumor cells are spread to distant sites via blood or lymphatic vessels. Extravasation through the walls of these vessels leads to the proliferation of a growth, a metastasis, at a site distant from the place of origin of the primary tumor. It has been proposed that only a few of the subpopulations of a developing tumor have metastatic potential (Fidler, 1988; Kerbel et al., 1988). The spreading process is also thought to be inefficient with most metastatic cells being destroyed by the body's immune defenses or as a result of mechanical/nutritional damage during circulation. Furthermore, metastases are not found at random suggesting that certain cancers metastasize preferentially to certain sites. Both the properties of the cells as well as the microenvironment into which they migrate are thought to be important in the spreading of cancer.

2.8.4 Mechanism of Neoplastic Progression

Mechanisms involved in the process of neoplastic progression appear to be varied. Genotoxic agents appear to affect progression by increasing the conversion of cells in a growth from benign to malignant properties (Yuspa and Poirier, 1988). Such conversions have been demonstrated with both chemical-induced (Colburn et al., 1987) and radiation-induced tumors (Jaffe et al., 1987). Oncogenes have been implicated not only in the initiation step, but also in the later steps in the oncogenic process (see Fig. 2.6; Farber, 1984). As already noted, the oncogene product transin is a protease found in malignancies derived from benign papillomas. Oncogenes that code for protein kinases have been shown to confer metastatic potential on fibroblastic cell lines (Seemayer and Cavenee, 1989).

Studies with chick embryos have demonstrated that both *src* and *ras* oncogenes can contribute to increased metastatic ability. In the latter example, the increased expression of the gene products correlated with increased malignancy (Chambers and Tuck, 1988). Changes induced by the *ras* gene include altered membrane glycoproteins, increased protease activity (including Type IV collagenase and plasminogen activator), increased growth factor secretion, increased motility, and increased invasiveness and metastatic ability.

In addition to specific genetic effects, other factors could play a role in progression. A lack of a response from the immune system to specific tumor-related antigens of the neoplastic cells could be important (Wheelock and Robinson, 1983). Because most solid neoplasms are highly vascularized, factors affecting angiogenesis can be expected to have a significant influence on the growth of a primary neoplasm as well as on its spread.

Although the pathophysiology of metastasis is well documented in a number of instances, the molecular changes that support the process are less clear and are a subject of active current research. The steps sketched in Fig. 2.6 are probably correct in general terms but the details for specific neoplasms can be expected to vary from one type of malignant growth to another.

2.9 SUMMARY

In the past 10-15 years, significant technical innovations have been introduced into the study of the cellular and molecular properties of mammalian cells including human cells. To the methods for the growth of mammalian cells in culture, which were introduced some 15 years earlier, genetic engineering/DNA recombination technology were added. As a result, it has become feasible to address fundamental questions in biology at a molecular level not achievable in the past. One such question has been the molecular/genetic basis of uncontrolled cellular growth, cancer.

In operational terms, neoplasias are believed to be the result of the three principal steps initiation, promotion, and *progression*. In Task 2, these steps are described in terms of the basic molecular and cellular processes that are responsible for them. Because a tumor results from one or more heritable changes in the progenitor cells of the tissue of origin, the mutation of genes called oncogenes is identified as a driving force in the development of cancer. Radiation, like other genotoxic agents, is known to damage DNA and is recognized as a mutagen/carcinogen. However, cells have the ability to modify genetic

damage and, consequently, repair/misrepair processes are important in the expression of the changes that are induced.

Because uncontrolled cellular growth is the feature that unifies neoplasms, a number of different genetic changes have been identified for their ability to play a role in the process. These changes occur in a set of normal genes, called proto-oncogenes, whose connection with neoplastic growth was first identified because of their involvement with the tumorigenic and molecular properties of RNA-containing viruses called retroviruses. The constitutive forms of the some 50 proto-oncogenes that have been identified have normal roles to play in cells. But when they are altered, as in the instance of a mutated form incorporated into the genome of a cell via a retrovirus, they can lead to uncontrolled cellular growth, or to a lack of control of differentiation, such that a neoplasm develops. Radiation, and other physical and chemical agents, is also able to mutate oncogenes and thus to give rise to the chain of events that culminate in cancer.

Through the application of DNA recombinant technology, a number of alterations in proto-oncogenes, or in the control of their expression, have been associated with the neoplastic transformation of cells. Hence, a variety of molecular mechanisms can culminate in an aberrant regulation of growth. As noted in Section 2.2, although examples exist of the association of neoplastic transformation with a mutation in a single p-oncogene, there is evidence that several altered genes may collaborate in the transformation/progression process involved in tumorigenesis (Weinstein *et al.*, 1984; Bishop, 1987; Weinberg, 1989). For example, it has been proposed that as many as 10 alterations may be involved in small-cell carcinoma of the lung.

Proto-oncogenes, like other genes, are transcribed into RNA from which a messenger form is produced that is then translated into proteins including the enzymes that catalyze various reactions. The products of proto-oncogenes, the proteins, have a variety of functions; they have been demonstrated to influence growth by controlling processes in other cells, or by acting in the cell of origin at its outer membrane, in its cytoplasm, or in its nucleus. The products of oncogenes are also known to influence the communication between adjacent cells changing thereby the societal properties of a cell.

Oncogene changes are also thought to be involved in the steps of *promotion* and *progression*. Promotion involves the clonal expansion of a transformed cell, and progression is associated with the ability of transformed cells to invade other tissues and to metastasize. Because radiation-induced neoplasms are indistinguishable from those that arise spontaneously, or are induced by some other etiological agent, and because of the mutagenic properties of radiation, it is likely that it can affect all of the steps in neoplasia. It is also possible, therefore, that the effects of radiation may interact with those of other environmental agents.

2.10 REFERENCES

- Adams, J. M., A. W. Harris, C. A. Pinkert, et al., "The c-myc Oncogene Driven by Immunoglobulin Enhancers Induces Lymphoid Malignancy in Transgenic Mice," Nature 318, 533-538, 1985.
- Ali, I. U., R. Liderau, C. Theillet, and R. Callahan, "Reduction to Homozygosity of Genes of Chromosome 11 in Human Breast Neoplasm," Science 238, 185-187, 1987.
- Ames, B.N., W. E. Durston, E. Yamasaki, and F. D. Lee, "Carcinogens Are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection," Proceedings of the National Academy of Sciences of the USA, 70, 2281-2283, 1973.
- Atkinson, M. M., A. S. Menko, R. G. Johnson, J. R. Sheppard, and J. D. Sheridan, "Rapid and Reversible Reduction of Junctional Permeability of Cells Infected with a Temperature-Sensitive Mutant of Rous Sarcoma Virus," Journal of Cell Biology 91, 53-64, 1981.
- Azarnia, R., S. Reddy, T. E. Kmiecik, D. Shalloway, and W. R. Loewenstein, "The Cellular src Gene Product Regulates Junctional Cell-to-Cell Communication," Science 239, 398-401, 1988.
- Barbacid, M., "Ras Genes," Annual Review of Biochemistry 56, 779-827, 1987.
- Barbacid, M., "Oncogenes and Human Cancer: Cause or Consequence?" Carcinogenesis 7, 1037-1042, 1986.
- BEIR III Report, "The Effects on Populations of Exposure to Low Levels of Ionizing Radiation," BEIR III, National Academy Press, Washington, D.C., 1980.
- BEIR V Report, "Health Effects of Exposure to Low Levels of Ionizing Radiation." National Research Council, Washington, D.C., 1990.
- Benedict, W. F., E. S. Srivatson, C. Mark, A. Banerjee, R. S. Sparkes, and A. L. Murphree, "Complete or Partial Homozygosity of Chromosome 13 in Primary Retinoblastoma," Cancer Research 47, 4189-4191, 1987.
- Bishop, J. M., "Cancer Genes Come of Age," Cell 32, 1018-1020, 1983a.
- Bishop, J. M., "Cellular Oncogenes and Retroviruses," Annual Review of Biochemistry 52, 301-354, 1983b.
- Bishop, J. M., "The Molecular Genetics of Cancer," Science 235, 305-311, 1987.
- Blanchard, J.-M., M. Piechaczyk, C. Dani, J.-C. Chambard, A. Franchi, J. Pouyssegur, and P. Jeantur, "c-myc Gene is Transcribed at a High Rate in G₀-arrested Fibroblasts and is Post-Transcriptionally Regulated in Response to Growth Factors," Nature 317, 443-445, 1985.

- Bridges, B.A. and Harnden, D.G. Ataxia-Telangiectasia: A Cellular and Molecular Link between Cancer, Neuropathology, and Immune Deficiency, Wiley & Sons, New York, 1982.
- Brinster, R. L., H. Y. Chen, A. Messing, T. Van Dyke, A. J. Levine, and R. D. Palmiter, "Transgenic Mice Harboring SV40 T-Antigen Genes Develop Characteristic Brain Tumors." Cell 37, 367-379, 1984.
- Brodeur, G., A. A. Green, F. A. Hayes, K. J. Williams, D. L. Williams, and A. A. Tsiatis, "Cytogenetic Features of Human Neuroblastomas and Cell Lines," Cancer Research 41, 4678-4686, 1981.
- Buckley, I., "Oncogenes and the Nature of Malignancy," Advances in Cancer Research 50, 71-94, 1988.
- Cavenee, W. K., T. P. Dryja, R. A. Phillips, W. F. Benedict, R. Godbout, B. L. Gallie, A. L. Murphree, L. C. Strong, and R. L. White, "Expression of Recessive Alleles by Chromosomal Mechanisms in Retinoblastoma," Nature 305, 779-784, 1983.
- Cavenee, W. K., "Loss of Heterozygosity in Stages of Malignancy." Clinical Chemistry 35/7(B), B48-B52, 1989.
- Chambers, A. F., and A. B. Tuck, "Oncogene Transformation and the Metastatic Phenotype," Anticancer Research 8, 861-872, 1988.
- Cleaver, J. E., "DNA Repair in Man." In: Genetic Susceptibility to Environmental Mutagens and Carcinogens, eds., A.D. Bloom and L. Spatz, March of Dimes Foundation, White Plains, New York, pp. 61-85, 1989.
- Colburn, N. H., E. Farber, I. B. Weinstein, L. Diamond, and T. J. Slaga, "American Cancer Society Workshop Conference on Tumor Promotion and Antipromotion," Cancer Research 47, 5509-5513, 1987.
- Cole, M. D., "The *myc* Oncogene: Its Role in Transformation and Differentiation," Annual Review of Genetics 20, 361-384, 1986.
- Cole, M. D., "Regulation and Activation of *c-myc*," Nature 318, 510-511, 1985.
- Collins, A.R. and Johnson, R.T., "DNA Repair Mutants in Higher Eukaryotes," Journal of Cell Science, Supplement 6, 61-82, 1987.
- Comings, D. E., "A General Theory of Carcinogenesis," Proceedings of the National Academy of Sciences of the USA 70, 3324-3328, 1973.
- Cornforth, M.N. and Bedford, J.S., "On the Nature of a Defect in Cells from Individuals with Ataxia-Telangiectasia," Science, 227, 1589-1591, 1985.
- Croce, C. M., "Chromosome Translocations and Human Cancer." Cancer Research 46, 6019-6023, 1986.
- De La Pena, P., and M. Zaslloff, "Enhancement of mRNA Nuclear Transport by Promoter Elements," Cell 47, 1199-1220, 1987.

- Doll, R. and R. Peto, The Causes of Cancer, Oxford University Press, Oxford, 1981.
- Elkind, M. M., "Repair Processes in Radiation Biology." Radiation Research 100, 425-449, 1984.
- Elkind, M. M., "Cells, Targets, and Molecules in Radiation Biology." In: Radiation Biology in Cancer Research, eds., R. E. Meyn and H. R. Withers, Raven Press, New York, pp. 71-93, 1980.
- Elkind, M. M., H. Utsumi, and E. Ben-Hur, "Are Single or Multiple Mechanisms Involved in Radiation-Induced Mammalian Cell Killing?" British Journal of Cancer 55, Suppl. VIII, 24-31, 1987.
- El-Fouly, M. H., J. E. Trosko, and C. C. Chang, "Scrape-Loading and Dye Transfer. A Rapid and Simple Technique to Study Gap Junctional Intercellular Communication," Experimental Cell Research 168, 422-431, 1987.
- Environmental Health Institute, Genetic Susceptibility to Environmental Mutagens and Carcinogens, Pittsfield, Massachusetts, 1989.
- Farber, E., "Pre-cancerous Steps in Carcinogenesis; Their Physiological Adaptive Nature," Biochimica et Biophysica Acta 738, 171-180, 1984.
- Fearon, R., A. P. Feinberg, S. H. Hamilton, and B. Vogelstein, "Loss of Genes on the Short Arm of Chromosome 11 in Bladder Cancer," Nature 318, 377-380, 1985.
- Fidler, I. J., "Origin of Cancer Metastases and its Implications for Therapy," Israel Journal of Medical Sciences 14, 456-463, 1988.
- Fisher, R. J., J. P. Bader, and T. S. Papas, "Oncogenes and the Mitogenic Signal Pathway," in Important Advances in Oncology (United States), pp 3-27, 1989.
- Friedberg, E.C., DNA Repair, W.H. Freeman & Co. New York, pp. 375-504, 1985.
- Furth, M. and Greaves, M., eds., Cancer Cells: Molecular Diagnostics of Human Cancer, Cold Spring Harbor Laboratory Press, New York, 1989.
- Friedberg, E. C., DNA Repair, Freeman & Co., New York, pp. 375-504, 1985.
- German, J., ed., Chromosome Mutation and Neoplasia Alan R. Liss, New York, 1983.
- Goustin, A. S., E. B. Leof, G. D. Shipley, and H. L. Moses, "Growth Factors and Cancer", Cancer Research 46, 1015-1029, 1986.
- Haluska, F. G., Y. Tsujimoto, C. M. Croce, "Oncogene Activation by Chromosome Transformation in Human Malignancy," Annual Review of Genetics 21, 321-345, 1987.
- Hanahan, D., "Dissecting Multistep Tumorigenesis in Transgenic Mice," Annual Review of Genetics 22, 479-519, 1988.

- Hanahan, D., "Transgenic Mice as Probes into Complex Systems." Science 246, 1265-1274, 1989.
- Hanawalt, P. C., "Intragenomic Heterogeneity in DNA Damage Processing: Potential Implications for Risk Assessment." In Mechanisms of DNA Damage and Repair, eds., M. Simic, L. Grossman, and A. Upton, Plenum Press, New York, pp. 489-498, 1986.
- Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith, "DNA Repair in Bacteria and Mammalian Cells," Annual Review of Biochemistry 48, 783-836, 1979.
- Harris, H., O. J. Miller, G. Klein, P. Worst, and T. Tachibana, "Suppression of Malignancy by Cell Fusion", Nature 223, 363-368, 1969.
- Jaffe, D. R., J. F. Williamson, and G. T. Bowden, "Ionizing Radiation Enhances Malignant Progression of Mouse Skin Tumors," Carcinogenesis 8, 1753-1755, 1987.
- Johnson, P. F. and S. L. McKnight, "Eukaryotic Transcriptional Regulatory Proteins." Annual Reviews of Biochemistry 58, 799-839, 1989.
- Jones, P. A., "DNA Methylation and Cancer." Cancer Research 46, 461-466, 1986.
- Kerbel, R. S., C. Waghorne, C. Korczak, A. Lagarde, and M. Brietman, "Clonal Dominance of Primary Tumors by Metastatic Cells: Genetic Analysis and Biological Implications," Cancer Surveys (England) 7 (4), 597-629, 1988.
- Klein, G., and E. Klein, "Evolution of Tumors and the Impact of Molecular Oncology," Nature 315, 190-195, 1985.
- Klein, G., "The Approaching Era of Tumor Suppressor Genes," Science 238, 1539-1545, 1987.
- Knudson, A. G., Jr., "Mutation and Cancer: Statistical Study of Retinoblastoma," Proceedings of the National Academy of Sciences of the USA 68, 820-823, 1971.
- Knudson, A. G., "Retinoblastoma: a Prototypic Hereditary Neoplasm," Seminars in Oncology 5, 57-60, 1978.
- Knudson, A. G., Jr., "The Genetic Predisposition to Cancer." Birth Defects (United States) 25, 15-27, 1988.
- Knudson, A. G., Jr., "Genetics of Human Cancer," Annual Review of Genetics 20, 231-251, 1986.
- Knudson, A. G., "Hereditary Cancer, Oncogenes, and Antioncogenes," Cancer Research 45, 1437-1443, 1985.
- Koufos, A., M. F. Hansen, B. C. Lampkin, M. L. Workman, N. G. Copeland, N. A. Jenkins, and W. K. Cavenee, "Loss of Alleles at Loci on Human Chromosome 11 During Genesis of Wilms's Tumor," Nature 309, 170-172, 1984.

- Krieg, P., J. Finch, G. Fustenberger, K. Melber, L. M. Matrisian, and G.T. Bowden, "Tumor Promoters Induce a Transient Expression of Tumor-associated Genes in Both Basal and Differentiated Cells of the Mouse Epidermis," Carcinogenesis 9, 95-100, 1988.
- Land, H., A. C. Chen, J. P. Morgenstern, L. F. Parada, and R. A. Weinberg, "Behavior of *myc* and *ras* Oncogenes in Transformation of Rat Embryo Fibroblasts," Molecular and Cellular Biology 6, 1917-1925, 1986.
- Land, H., L. F. Parada, R. A. Weinberg, "Cellular Oncogenes and Multistep Carcinogenesis," Science 222, 771-778, 1983.
- LeBeau, M.M. and Rowley, J.D., "Chromosomal Abnormalities in Leukemia and Lymphoma: Clinical and Biological Significance," In: Advances in Human Genetics, eds., H. Harris and K. Hirshhorn, Plenum Publishing Co., New York, 15, 1-54, 1986.
- Levinson, A. D., "Normal and Activated *ras* Oncogenes and their Encoded Proteins," Trends in Genetics 2, 81-85, 1986.
- Lijinsky, W., "A View of the Relation between Carcinogenesis and Mutagenesis," Environmental and Molecular Mutagenesis 14, Supplement 16, 78-84, 1989.
- Liotta, L. A., "Tumor Invasion and Metastases: Role of the Basement Membrane," American Journal of Pathology 117, 339-348, 1984.
- Lundberg, C., L. Skoog, W. F. Cavenee, and M. Nordenskjold, "Loss of Heterozygosity in Human Ductal Breast Tumors Indicates a Recessive Mutation on Chromosome 13," Proceedings of the National Academy of Sciences of the USA 84, 2372-2376, 1987.
- Marks, F., "What's New in Oncogenes and Growth Factors?" Pathology Research and Practice 182, 831-848, 1987.
- Matrisian, L. M., G. T. Bowden, P. Krieg, G. Furstenberger, J. P. Briand, P. Leroy, and R. Breathnach, "The mRNA Coding for the Secreted Protease Transin is Expressed More Abundantly in Malignant than in Benign Tumors," Proceedings of the National Academy of Sciences of the USA 83, 9413-9417, 1986.
- Mehta, P. P., J. S. Bertram, and W. R. Lowenstein, "Growth Inhibition of Transformed Cells Correlates with Their Junctional Communication with Normal Cells," Cell 44, 187-196, 1986.
- Mellon, I., Spirak, G., and Hanawalt, P.C. "Selective Removal of Transcription-Blocking DNA Damage from the Transcribed Strand of the Mammalian DHFR Gene," Cell 51, 241-249, 1987.
- Miller, E. C., and J. Miller, "The Mutagenicity of Chemical Correlations, Problems, and Interpretations." In: Chemical Mutagens: Principles and Methods for Detection. ed., A. Hollaender, vol. 1, pp. 83-113, 1971.
- Mukai, S., and T. P. Dryja, "Loss of Alleles at Polymorphic Loci on Chromosome 2 in Uveal Melanoma," Cancer Genetics and Cytogenetics 22, 45-53, 1986.

National Research Council, Identifying and Estimating the Genetic Impact of Chemical Mutagens, National Academy Press, Washington, D. C., 1983.

Nowell, P. C. and C. M. Croce, "Chromosomes, Genes and Cancer," American Journal of Pathology 125, 8-15, 1986.

Office of Technology Assessment, OTA-H-298, Technologies for Detecting Heritable Mutations in Human Beings, U. S. Government Printing Office, Washington, D. C., 1986.

Painter, R.B., "Structural Changes in Chromatin as the Basis for Radiosensitivity in Ataxia Telangiectasia", Cytogenetics and Cell Genetics, 33, 119-132.

Pathak, S., L.C. Strong, R. E. Ferrell, and A. Trindade, "Familial Renal Cell Carcinoma with a 3:11 Chromosome Translocation Limited to Tumor Cells," Science 217, 939-941, 1982.

Pearson, M. and J. D. Rowley, "The Relation of Oncogenesis and Cytogenetics in Leukemia and Lymphoma," Annual Review of Medicine 36, 471-483, 1985.

Pienta, A.J., Partin, A.W., and Coffee, D.S. "Cancer as a Disease of DNA Organization and Dynamic Cell Structure," Cancer Research 49, 2525-2532, 1989.

Rowley, J. D., "Biological Implications of Persistent Chromosome Rearrangements in Leukemia and Lymphoma," Cancer Research 44, 3159-3168, 1984.

Sager, R., "Tumor Suppressor Genes: The Puzzle and the Promise." Science 246, 1406-1412, 1989.

Sankaranarayanan, K., Genetic Effects of Ionizing Radiation in Multicellular Eukaryotes and the Assessment of Genetic Radiation Hazards in Man, Elsevier, Amsterdam, 1982.

Seemayer, T. A., and W. K. Cavenee, "Biology of Disease: Molecular Mechanisms of Oncogenesis," Laboratory Investigation 60, 585-599, 1989.

Seizinger, B. R., R. L. Martuza, and J. F. Gusella, "Loss of Genes on Chromosome 22 in Tumorigenesis of Human Acoustic Neuroma," Nature 322, 644-647, 1986.

Sinkovics, Joseph G., "Oncogenes and Growth Factors," CRC Critical Review in Immunology 8, 217-298, 1988.

Solomon, E., R. Voss, V. Hall, W. F. Bodmer, J. R. Jass, A. J. Jeffreys, F. C. Lucibello, I. Patel, and S. H. Rider, "Chromosome 5 Allele Loss in Human Colorectal Carcinomas," Nature 328, 616-619, 1987

Stamato, T. D., L. Hinkle, A. Collins, C. Waldren, "Chinese Hamster Ovary Mutant UV-1 is Hypomutable and Defective in a Postreplication Recovery Process," Somatic Cell and Molecular Genetics 7, 307-320, 1981.

Stanbridge, E. J., "Genetic Analysis of Human Malignancy Using Somatic Cell Hybrids and Monochromosome Transfer," Cancer Surveys 7, 317-324, 1988.

- Stewart, T. A., P. Pattengale, P. Leder, "Spontaneous Mammary Adenocarcinomas in Transgenic Mice that Carry and Express MTV/myc Fusion Genes," Cell 38, 627-637, 1984.
- Swift, M., Reitnauer, P.J., Morrells, D., and Chase, C.L., "Breast and Other Cancers in Families with Ataxia Telangiectasia," The New England Journal of Medicine 316, 1289-1294, 1989.
- Tabin, C. J., S. M. Bradley, C. O. Bargmann, R.A. Weinberg, A. G. Papageorge, E. Scolnick, R. Dhar, D. R. Lowy, and E. H. Chang, "Mechanism of Activation of a Human Oncogene," Nature 300, 143-152, 1982.
- Tannock, I.F. and Hill, R.P., eds., The Basic Science of Oncology, Pergamon Press, New York, 1987.
- Thacker, J., "The Nature of Mutants Induced by Ionizing Radiation in Cultured Hamster Cells. III. Molecular Characterization of the HPRT-Deficient Mutants Induced by γ -Rays or α -Particles Showing That the Majority Have Deletions of All or Part of the HPRT Gene," Mutation Research 160, 267-275, 1986.
- Varmus, H. E., "The Molecular Genetics of Cellular Oncogenes." Genetics 18, 553-612, 1984.
- Vijayalaxmi, H. J. Evans, J. H. Ray, J. German, "Bloom's Syndrome. Evidence for an Increased Mutation Frequency In Vivo," Science 221, 851-852, 1983.
- Walker, G. C., "Inducible DNA Repair Systems," Annual Review of Biochemistry 54, 425-457, 1985.
- Weinberg, R. A., "Oncogenes, Antioncogenes, and the Molecular Bases of Multistep Carcinogenesis," Cancer Research 49, 3713-3721, 1989.
- Weinstein, I. B., S. Gattoni-Celli, M. Krischmeier, M. Lambert, W. Hsiao, J. Backer, and A. Jeffrey, "Multistage Carcinogenesis Involves Multiple Genes and Multiple Mechanisms," in Cancer Cells 1. The Transformed Phenotype, eds., A. J. Levine, G. F. Van de Woude, W. C. Topp, and J. D. Watson, Cold Spring Harbor Laboratory, New York, pp 229-237, 1984.
- Whang-Peng, J., P. A. Bunn, Jr., C. S. Kao-Shan, E. C. Lee, D. N. Carney, A. Gazdar, and J. A. Minna, "A Nonrandom Chromosomal Abnormality, del 3p(14-23), in Human Small Cell Lung Cancer (SCLC)," Cancer Genetics and Cytogenetics 6, 119-134, 1982.
- Wheelock, E. F., and M. K. Robinson, "Endogenous Control of the Neoplastic Process," Laboratory Investigation 48, 120-139, 1983.
- Willis, A.E., Weksberg, R., Tomlinson, S., and Lindahl, T., 1987, "Structural Alterations of DNA Ligase I in Bloom Syndrome." Proceedings of the National Academy of Sciences, USA 84, 8016-8020, 1987.
- Witkin, E., "Ultraviolet Mutagenesis and the SOS Response in *Escherichia Coli*: A Personal Perspective." Environmental and Molecular Mutagenesis 14, Supplement 16, 23-29, 1989.

Yamasaki, H., and D. J. Fitzgerald, "The Role of Selective Junctional Communication and Tumor Promoter-Inhibited Communication in Cell Transformation." In: Tumor Promoters, eds, R. Langenbach, C. J. Barrett, and J. Elmore, Raven Press, New York, pp. 131-147, 1988.

Yunis, J. J., "The Chromosomal Basis of Human Neoplasia." Science 221, 227-236, 1983.

Yuspa, S. H. and M. C. Poirier, "Chemical Carcinogenesis: From Animal Models to Molecular Models in One Decade." Advances in Cancer Research 50, 25-69, 1988.

Task 3

"Elements of a Radiation Research Program" *Cellular/Molecular Mechanisms in Cancer Induction*

3.1 INTRODUCTION

During the past 50 years, an extensive literature has developed in radiobiology to which contributions have come from scientists in many countries. Studies were performed dealing with the physical, physical chemical, and biochemical consequences of radiant energy absorption as well as with radiation effects in cells, tissues, and animals. However, in the past decade, the advent of new molecular techniques--particularly those that facilitate the study of the genotypic changes connected with biological endpoints like radiation-induced lethality, mutation, neoplastic transformation, and tumor induction--have raised the prospect that the molecular biology of these effects could be understood.

The initial objective of this Task is to evaluate the *in vitro* and *in vivo* data that are available in the context of the mechanism(s) that underlies unregulated growth induced by radiation. Although the principal focus will be on the effectiveness of low doses of protracted low-LET radiations--that is, the radiations to which most people are exposed--results obtained with radiations of higher LET will also be discussed where they may facilitate an understanding of mechanisms. The second objective is to discuss oncogene action in neoplasia as a basis for identifying gaps in the understanding of the role of oncogenes in radiation-induced cancer.

It is generally believed that heritable changes introduced into the genomic DNA of somatic cells are responsible for unregulated growth and, therefore, for neoplasia. Indeed, as the material in this report makes clear, the evidence indicates that cancer is the culminating phenotypic expression of one or more genetic changes. Heritable effects in general, however, will not receive detailed attention. First, as illustrated by the data reviewed in various reports like the BEIR V Report (1990), the lifetime risk of radiation-induced cancer is considered to be appreciably greater than that of radiation-induced heritable changes in germ-line cells. Hence, permissible dose limits are based primarily on the former endpoint rather than on the latter. And second, heritable physiological anomalies, which may be attributable to radiation exposure, are not likely substantially to influence radiation susceptibility to cancer.

Although, as noted above, the broad range of possible genetic effects are not germane to this study, an important exception is mutation in somatic cells. As discussed in Sections 2.2, 2.3, and 3.3, somatic mutation is considered to be the principal molecular starting point for the initiation of the phenotypic changes that culminate in neoplastic growth.

3.2 RADIATION-INDUCED NEOPLASTIC TRANSFORMATION, *IN VITRO* vs. *IN VIVO* DATA

During the past 15 years, results with animal cells in culture have formed a basis for examining the molecular and cellular mechanism of radiation-induced tumorigenesis. In this Section, these results are reviewed and critically analyzed.

3.2.1 Neoplastic Transformation. Cells in Culture

Because of technical problems in developing suitable assay systems, quantitative data on the induction of neoplastic transformation in mammalian cells has been limited largely to results obtained with three kinds of rodent cells: C3H mouse embryonic fibroblasts, designated 10T1/2 (Reznikoff *et al.*, 1973); BALB/c mouse fibroblasts designated 3T3 (see Little, 1979); and Syrian hamster embryo cells (see Borek and Sachs, 1966). Still, a number of results have been obtained which show that qualitative agreement exists in a number of instances between the data obtained with these rodent cells *in vitro* and tumor induction *in vivo*. With 10T1/2 and 3T3 cells, the assay of cells that are phenotypically transformed is based upon the (infrequent) appearance of morphologically-altered foci of cells growing in a monolayer of normal cells. These altered cells, but not their normal counterparts, are capable of forming fibrosarcomas at the site of inoculation into suitable hosts. With Syrian hamster embryo cells, the transformed state is detected as an (infrequent) morphologically altered discrete colony which, in contrast to its normal counterpart, is capable of producing a tumor at the site of inoculation into suitable hosts.

3.2.1.1 Induction of Transformation vs. killing

Using 10T1/2 cells, Han *et al.* (1980) determined that the frequency of transformation was a product of the frequency of induction of the transformed phenotype and the probability of survival of the initiated cell; a dose dependence of survival that was found to be essentially the same as for noninitiated cells. They also showed the damage, which led to transformation, was repairable at reduced dose rates (see Subsection 3.2.1.2), and this repair was distinct from the repair of sublethal damage (Elkind and Sutton, 1959).

3.2.1.2 Dependence on Dose Rate and Dose Protraction

The results of Han and Elkind (1979) and Han *et al.* (1980) are summarized in schematic form in Fig. 3.1 (see legend). The bell-shaped dashed curve in the lower left panel (repeated in the lower right panel) is the frequency of transformation per cell exposed for high dose rates of ^{60}Co γ -rays (linear coordinates). The large effect of cell killing was made evident by normalizing the latter results by the surviving fraction as a function of dose to yield the upper dashed curves marked "high D' (-killing)," that is, the frequency per surviving cell. With 10T1/2 cells, the absolute frequency of transformants maximizes at a dose of 4-6 Gy (Han *et al.*, 1980). If only the repair of sublethal damage influenced the frequency of transformation, the curve "low D'" in the lower left panel would have been expected indicating that a higher transformation frequency at all doses would be observed at a low dose rate. The curve marked "low D'" in the lower right panel, in fact, was observed.

Hence, the results sketched in Fig. 3.1 showed that in an assay system based upon a single cell type, as opposed to the situation in an animal, two repair processes influenced the yield of transformants: 1) the repair of sublethal damage (which permits a larger number of transformed cells to be expressed); and 2) the repair of subtransformation (or subeffective) transformation damage. The net result was that, in the region of small doses, the induction frequency that was observed at a low dose rate--or with the protraction of doses delivered at a high dose rate (Hill *et al.*, 1984)--was lower than the induction frequency observed at a high dose rate (see Elkind *et al.*,

Fig. 3.1 The dependence of cell survival (upper panel) and neoplastic transformation (lower panels) on dose rate of X-rays. The upper panel shows the upward shift of the survival curve of 10T1/2 cells when they were irradiated at a reduced dose rate. In the lower left panel (linear coordinates), the lower dashed curve is the frequency of neoplastic transformants of 10T1/2 cells irradiated at a high dose rate; therefore, this curve includes the effect of cell killing. The upper dashed curve in this panel is the induction curve that would have been observed if the lethal effect of the high-dose-rate exposure was factored out. For a given dose, a reduced dose rate allows more cells to survive; hence, the continuous curve in the lower left panel shows what would have been expected in only the repair of sublethal damage had influenced the net transformation frequency. The transformation frequency that was actually observed is shown in the right lower panel. (From Han et al., 1980.)

1985). In the region of high doses, the reverse was the case because the large sector of survival sparing that was effected by the repair of sublethal damage could increase the yield of transformants sufficiently to more than compensate for the reduction in the yield due to the repair of subeffective transformation damage.

In general, the cellular data for the protraction of two or more high-dose-rate exposures have indicated that reduced transformation frequencies are obtained (e.g., Hill et al., 1984). Although some results suggested that an increase in frequency resulted when a specific interfraction interval is used, 5 hours, and total doses less than 1.5 Gy (Miller and Hall, 1978; Borek, 1979; Little, 1979), confirmation of such an enhancement in transformation frequency *in vitro*, or in tumorigenesis, was not confirmed by other results. That is, the studies of Yang et al., (1986) and Balcer-Kubiczek et al., (1987) with 10T1/2 cells confirmed that reducing the dose rate reduced the yield of transformants as did similar studies with Syrian hamster embryo cells (Watanabe et al., 1984).

Hill et al., (1987, 1989) showed further that the slope of the induction curve became progressively less steep as the dose rate was decreased. However, the latter studies also revealed that a signal remained in exposed cells that could be amplified by a chemical promoter such as a phorbol ester. Thus, although one could infer that the slope of the induction curve might approach zero at dose rates encountered in the environment, it remains a possibility that a second environmental agent might be able to increase the slope of the curve because the radiation can register a change in the target cells that does not disappear as completely as the subeffective damage due to the radiation alone.

3.2.1.3 Dependence on Radiation Quality

Several sets of measurements have been made with high-LET radiations like fast neutrons (see Han and Elkind, 1979) or charged atomic particles (Yang et al., 1986). Because LET is a radiobiological parameter of importance for many endpoints, results with high-LET radiations are briefly discussed to indicate where qualitative internal consistencies exist between the endpoints neoplastic transformation *in vitro* and tumor induction, and where they do not.

In general, with increasing LET endpoints are produced with increasing effectiveness per unit dose until a maximum is reached between about 100-200 KeV/ μ m (see Elkind and Whitmore, 1967; Upton, 1988). The latter trend applies to cell killing, somatic mutation, neoplastic transformation, life shortening, and tumor induction although the full range of LET's are not available for every endpoint. However, some additional features of the dependence on LET are illustrated in Fig. 3.2. Han and Elkind (1979) showed with 10T1/2 cells that the incidence of transformants with dose induced by fission-spectrum neutrons traced a bell-shaped curve (linear coordinates) as was also the case for 50 KV X-rays and ^{60}Co γ -rays (Han et al., 1980). That is, the expression of the induction of transformants was attenuated progressively with dose by cell killing producing a maximum in the curve. As was the case for X- and γ -rays, the survival response of the initiated cells was the same as that of the uninitiated cells. The net effect was a shift in the curve due to neutrons to smaller doses from the curve for γ -rays, or X-rays, and an increase in the maximum in the neutron curve compared to the maximum in the γ -ray curve.

In contrast to cellular studies with low-LET radiations, studies with tended exposures to some high-LET radiations in some instances have yielded

Fig. 3.2 The qualitative dependence of neoplastic transformation *in vitro* on the quality of the radiation when high-dose-rate exposures are used. In both cases, the bell-shape curve is due primarily to the lethal effect of the radiation on potentially transformed cells. (From Elkind, 1987; adapted from Han and Elking, 1979.)

enhanced frequencies of transformation (Hill *et al.*, 1982, 1984, 1985; Yang *et al.*, 1986; Jones *et al.*, 1989), and in other instances have not (Hieber *et al.*, 1987; Balcer-Kubiczek *et al.*, 1988). Because the same cells were used in several of the foregoing studies that either did or did not show an enhancement, it is likely that the lack of correlation is attributable to differences in the sources of radiation and/or techniques. This subject is discussed further in Subsection 3.2.3.

3.2.2 Neoplasia, Rodents and People

The data available on neoplasia induced by radiation comes mostly from studies with rodents. The dose dependencies for the induction of human cancer are generally less precise. As in the studies of experimental tumorigenesis with animals, the human data frequently refer to the irradiation of large portions of the body, if not the entire body (see Task 1). Consequently, in seeking mechanistic relationships between results with cells *vs.* those with animals and people, tissue interactions may play a role if the radiation is not confined to the tissue of interest. In addition, other types of interactions may exist in the induction and expression of neoplasia. In spite of these complexities, consistencies are apparent between *in vitro* and *in vivo* results.

3.2.2.1 Dose Dependencies, Induction *vs.* Killing

L.H. Gray (1965) was the first to propose that tumorigenesis is the net effect of a process of induction attenuated by a process of killing of the susceptible cells in the tissue in which the tumor arises. He found that the data for myeloid leukemia induction in mice (Upton *et al.*, 1970) were fit quite well by a concave-upward induction curve modified by a survival curve that had survival parameters typical of those determined with cultured cells. Gray's hypothesis was found to be applicable to other results on leukemia induction in mice (see Elkind *et al.*, 1983) as well as to an epidemiological analysis of the induction of leukemia, as second tumors, in women who had been treated with radiation for cancer of the cervix (Boice *et al.*, 1988). In the latter study, a bell-shaped induction curve was arrived at by accounting for the dose received by bone marrow cells at various sites in the body as well as the lethal effect of the radiation on the target cells of the bone marrow at those sites. Although the foregoing observations and analyses are consistent with Gray's hypothesis, the validity of his model has been strongly reinforced by studies of the influence of repair processes--that is, observations at low dose rate and with dose protraction--on cell transformation on the one hand, and tumor induction on the other.

3.2.2.2 Extended Exposures, Low Spontaneous Incidence

The framework for an understanding of the role of repair in neoplasia induced by a low-LET radiation comes from the studies of Han *et al.*, (1980) the essential results of which are sketched in Fig. 3.1. They found that extending sufficiently the length of an exposure, by reducing the dose rate or protracting a series of high-dose-rate exposures (Hill *et al.*, 1984), permitted two separate kinds of repair to operate; the repair of sublethal damage and the repair of subeffective transformation damage. As noted in Subsection 3.2.1.1, the latter process reduced the initial slope of the induction curve, whereas the former caused the frequency for large doses to be greater than it would be for a brief exposure. The net effect was to cause the high-dose-rate bell-shaped curve to broaden, and for its maximum to be reduced and shifted to higher doses. Examples

of reductions in the initial yields of mouse tumors of various kinds at reduced dose rates can be found in the study of Ullrich and Storer (1979), and similarly for mouse myeloid leukemia in the results of Upton et al. (1970). The induction of tumors of the hair follicle of the rat showed a bell-shaped induction curve, and in that study data for 2-dose protraction were consistent with the effects of both types of repair (Burns and Vanderlaan, 1977). In the case of patients who were treated with multiple doses of X-rays for ankylosing spondylitis, the induction of leukemia (Smith and Doll, 1982) indicates a dose response consistent with the effects of repair; that is, a collapse of the single-dose induction curve because of dose protraction.

3.2.2.3 Extended Exposures, High Spontaneous Incidence

For the most part, the data on experimental tumorigenesis with low-LET radiation indicate a reduction in the frequency in the initial part of the induction curve when the exposures are protracted. In the instance of reticulum cell sarcoma¹ in mice, however, Ullrich and Storer (1979) reported that the frequency at a low dose rate was greater than it was at a high dose rate. This apparent anomaly has been explained as consistent with, and indeed to be a reflection of, the influence of both the repair of sublethal damage and of subeffective damage when the cells responsible for the high spontaneous incidence were present at the time of exposure. More specifically, the explanation of the preceding inverse effect of dose rate, with a low-LET radiation, rests on these elements: 1) a high spontaneous rate of appearance of this tumor in the strain of mice that were used; 2) the presence of progenitor cells at the time of the high-dose-rate exposures; and 3) a sparing of killing of the latter cells at the reduced dose rate (see Elkind et al., 1983; Elkind, 1987).

The alternatives, in reference to the time of appearance of the progenitor cells of the spontaneous tumors, are sketched in Fig. 3.3 (Elkind, 1987). In the left panel, the lower pair of curves trace what is also shown in the lower right of Fig. 3.1. That is, reducing the dose rate causes the high-dose-rate curve to flatten and to shift its maximum toward higher doses. If, after the completion of the exposures, progenitor cells arise that are responsible for the spontaneous incidence, then the lower pair of curves would be shifted upward starting from the spontaneous frequency. In contrast, as shown in the right panel of Fig. 3.3, it is assumed that the progenitor cells of the spontaneous tumors are present at the start of the irradiations and are progressively killed at the high dose rate. As a result, as the dose is increased from zero, the high-dose-rate curve initially drops before it joins the lower curve due to the induction of tumors at a high dose rate in susceptible target cells, lower right of Fig. 3.3. In the limit of a very low dose rate and therefore no killing of the progenitor cells, the overall frequency of tumors would be the sum of the low-dose-rate induction curve (left panel) added to the spontaneous incidence.

As sketched in the right panel of Fig. 3.3, the net result of the sparing of the progenitor cells is that the incidence at a low dose rate lies above that at a high dose rate--as Ullrich and Storer (1979) reported for reticulum cell sarcoma--in contrast to what is ordinarily observed for X- or γ -ray tumorigenesis. Evidence supporting the foregoing can be found in the study of Metalli et al. (1978) who also induced reticulum cell sarcomas in mice in which

¹Reticulum cell sarcoma refers to a group of neoplasms of histiocytic and lymphomatous origin (Pattengale and Taylor, 1983).

γ-RAYS

Fig. 3.3 Left Panel: The effect of dose rate D' on the induction of tumors when the spontaneous incidence is low, lower curves; and when the spontaneous incidence is high, but the progenitor cells responsible for the spontaneous incidence are not present at the time of the high-dose-rate exposures, upper curves. Right Panel: In contrast to the left panel, the upper curves show the result when the spontaneous incidence is high and the progenitor cells responsible for the spontaneous incidence are present at the time of the high-dose-rate exposures. (From Elkind, 1987.)

the spontaneous rate was comparable to the rate in the mice used by Ullrich and Storer (1979). By shielding the hind limbs and exposing the rest of the animal to a large dose at a high dose rate, they reduced the frequency of tumors to a low level indicating that most of the progenitor cells were present at the time of exposure and that they were radiosensitive. Thereafter, graded high-dose-rate exposures applied to unshielded hind limbs induced tumors in the marrow that had been shielded to yield an incidence of tumors with dose that traced a typical bell-shaped curve similar to the high-dose rate curve in the lower left panel of Fig.3.3. Thus, the results of Metalli et al., (1978) illustrated that the progenitor cells of the spontaneous reticulum cell sarcoma, in fact, were present at the time of irradiation and that their sensitivity to killing was typical of that of hematopoietic stem cells (Elkind, 1987).

Therefore, the result obtained with reticulum cell sarcoma, which at first glance appears to be anomalous, is what would be expected if cell killing is important to the net yield of tumors, and if both the repair of sublethal and subeffective transformation damage play a role.

3.2.2.4 Tumorigenesis, Fission-Spectrum Neutrons

In experimental tumorigenesis, the dependence on radiation quality has been examined largely with fast neutrons mainly because of the limited penetration of beams of charged particles. Also, most of the work employed neutron beams produced by reactors. In general, neutrons were found to be more effective than low-LET radiations but yielded results qualitatively similar to those sketched for cellular transformation in Fig. 3.2. In the light of the revised dosimetry for the atomic bomb explosions at Hiroshima and Nagasaki in 1945, essentially no reliable data exist for neutron-induced human neoplasia.

3.2.2.5 Radiation-Induced Life Shortening

It is well established that the average life span of an animal is progressively reduced with increasing dose to the entire body (see BEIR V Report, 1990). Because the reduction due to small doses appears to be due to death caused by tumors, the endpoint *life shortening* has been considered to be an integrator of tumorigenesis in the region of small doses. Data have been developed on the effect of radiation on the life span of mice exposed to single and multiple weekly doses over extended periods.

An extensive study at the Argonne National Laboratory compared single and protracted exposures of ^{60}Co γ -rays (see Thomson et al., 1986, and the preceding papers in their series). The major features of the data, in reference to the influence of extended exposures, have been summarized by Upton (1988). Compared to single exposures, equal total doses delivered as multiple weekly or as low-dose-rate exposures, were appreciably less effective in shortening life span. Most of the deaths following low doses were due to tumorigenesis.

3.2.3 Low-Dose-Rate and Protracted Exposures, Fast Neutrons

Although it is generally observed that both *in vitro* as well as *in vivo* endpoints are reduced in signal strength when a low-LET dose is extended in time, mixed results have been reported when prolonged exposures of a high-LET radiation were used like low-dose-rate or protracted high-dose-rate exposures to fission-spectrum neutrons. To begin with, it was noted with cells in culture (Subsection 3.2.1.3) that enhanced transformation frequencies were observed in some but not

in all instances. A similar situation exists for tumorigenesis and for life shortening in mice.

3.2.3.1 The Endpoint Life Shortening, Protracted Exposures

Data obtained at the Argonne National Laboratory showed that single doses of neutrons were appreciably more effective than single doses of γ -rays in reducing life span (see Thomson et al., 1986). Also, larger losses in life span were observed when neutron doses were given in 24 or 60 weekly fractions compared to single exposures. In the dose range 0-20 cGy, a statistically significant difference between the data for single doses vs. protracted doses was not evident, but the data were not extensive enough to establish if the various kinds of neoplasms that were induced followed the same or different dose dependencies as for life shortening. Hence, relative to life shortening as a reflection of tumorigenesis, the apparent lack of a difference between protracted and single exposures in the low-dose region could have resulted from an increase in the yields of some tumor types that were offset by decreases in others.

3.2.3.2 The Endpoint Tumorigenesis, Low-Dose-Rate and Protracted Exposures

In respect to other neoplasms, Ullrich (1984) reported an increase in mammary carcinomas in mice exposed at a reduced dose rate of fission-spectrum neutrons with the effect already evident at 2.5 cGy. Enhancement was not observed at other sites. The enhanced effect of extended exposures of neutrons was also evident in the rate of progression toward neoplasia of mammary epithelial cells (Ullrich, 1986). In mice injected with ^{224}Ra , higher frequencies of osteosarcoma were observed when twice-weekly injections, spread out from 4 to 36 weeks, were used compared to single injections (Muller et al., 1978). Further, the use of this isotope to treat patients with ankylosing spondylitis caused more bone sarcoma when the exposure was protracted (Spiess and Mays, 1973). The induction of lung cancer among miners due to the α -particle-emitting progeny of radon also appears to demonstrate an enhancement at reduced exposure rates (Curtis, 1989). The data for α -particle irradiations may have reflected an increase in the availability of susceptible target cells, due to compensatory repopulation, when the exposures were protracted. But also they may be another indication of an increased tumorigenic effect of a high-LET radiation when the exposures were prolonged.

3.2.4 Gaps in *in Vitro/in Vivo* Knowledge

Although progress has been made since the advent of cellular systems for quantitating the effectiveness of radiation in inducing neoplastic transformation, neither a complete nor fully consistent picture has been developed. Gaps in our understanding and in the information required are reviewed below.

3.2.4.1 *In Vitro* Studies, Types of Cells

Quantitative data are needed, based upon endpoints indicative of neoplastic transformation, with additional lines of rodent fibroblasts in order to complete the pattern of dependencies on cellular parameters. Cells of epithelial origin are also needed because most human tumors originate in epithelium. Further, to facilitate extrapolation from rodents to man, dog, monkey, and human cells for transformation studies are needed. In addition to the establishment of the generality of responses relative to radiobiological parameters, cells from

different tissues and species would enable models to be tested for the role(s) of oncogenes in the process(es) of neoplastic transformation.

Related to the need to develop a more comprehensive understanding of neoplastic transformation and its dependence on radiobiological parameters is the understanding of how radiation kills cells and the molecular biology of how repair-competent cells mitigate the effects of lethal damage. During protracted exposures to radiation, the repair of sublethal damage (Elkind and Sutton, 1959) results in reduced killing. The molecular basis of such repair and its connection, if any, to the repair of preneoplastic lesions (Elkind et al., 1985) needs to be determined.

3.2.4.2 Rate of Exposure and the Interaction of Radiation with Other Agents

In reference to the biological and radiobiological parameters referred to in Subsections 3.4.4.1 & 2, the information is not complete on the dependence of transformation on exposure rate, radiation quality, and the effect of other physical agents (e.g., ultraviolet light) or chemical agents (e.g., mutagens/carcinogens, growth factors, and promoters and antipromoters). The combined effects of these factors will be more predictable as the molecular basis of neoplastic transformation becomes better understood.

In addition to the combined action of other agents with radiation, it is important to understand the dependence of transformation on exposure rate, and whether or not radiation quality, cell type, and species of origin influence the yields of transformation

3.2.4.3 Dose Dependencies *in Vivo*

Aside from the presence or absence in inbred rodents of progenitor cells at the time of exposure, the dose dependence of tumorigenesis varies qualitatively and quantitatively with the site of origin (see Ullrich, 1984, 1986; Ullrich and Storer, 1979; Ullrich et al., 1976). The role of cell killing is evident in the induction of some kinds of tumors, but because the data are limited it is not clear if susceptible target cells are all similarly affected. It is also not known whether at different sites tumorigenesis in out bred mice, or in other species, would be the same as that observed in inbred mice. Lastly, it is not known if and how tumor induction in different tissues depends on the length of exposure to radiations of LET higher than that of X- and γ -rays.

3.3 GENES AND ONCOGENES IN RADIATION-INDUCED ENDPOINTS

The development of the techniques of recombinant DNA for examining the properties of the genome, even at the level of nucleotide base sequences (see Burck et al., 1988), has facilitated the study of the roles of cancer genes in various radiation-induced endpoints. In addition to germ-cell mutations--which were referred to in Task 1, Section 1.6--other endpoints that have received attention have been cell killing/survival, somatic mutation, neoplastic transformation, and tumorigenesis. The involvement of genes, including cancer genes, in the foregoing endpoints is reviewed in this Section.

3.3.1 Cell Survival

As described below, several studies implicate certain oncogenes in the survival response of mammalian cells.

3.3.1.1 *c-raf* and Squamous-Cell Laryngeal Carcinoma

The radiotherapy of head and neck tumors has led to the conclusion that certain of these tumors are radioresistant. Kasid et al. (1987, 1989) used the technique of transfecting DNA into mouse 3T3 cells to demonstrate that the transfectants: 1) induced tumors at the site of inoculation in immune-deficient mice; 2) contained an altered *c-raf* gene; and 3) gave rise to a population of cells that were radioresistant. Their attribution of the latter property to an altered *c-raf* gene was supported by the observation that resistant transformed cells lost their resistance (final D_0 reduced from 3.1 to 1.9 Gy) after they were transfected with a plasmid containing an antisense region of the *c-raf* gene (Kasid et al., 1989). These secondary transfectants were significantly less tumorigenic in immune-deficient mice than the initial transfectants suggesting that the antisense mRNA, which was produced from the antisense DNA contained in the plasmid, inactivated the sense mRNA. These authors proposed that, as a result, less of the normal gene product of *c-raf* was produced thus causing the cells to be reverted toward normality.

3.3.1.2 The *ras* Oncogene and Radiation Resistance

As noted in Task 2, the genomic DNA from various tumors is able to transform 3T3 cells when the DNA is introduced by a transfection procedure (see Kucherlapati, 1986). Sklar (1988) used this approach to examine the dependence of the radiation survival properties of 3T3 transfectants on the nature of the mutation of various *ras* oncogenes that were used in the transfection. For comparison, transfections were also carried out with other human oncogenes. Sklar reported that missense mutations in *ras* (mutations that coded for an altered gene product of *ras*) conferred radioresistance on the 3T3 cells that he used (i.e., the final D_0 was increased from 1.4 to about 2.1 Gy). Similar increases were not observed when 3T3 cells were transfected with a normal *ras* gene or with the oncogene *v-fms*. He also found that cells, which reverted to a normal-appearing morphology, but which still contained functional *ras* oncogenes, were still radioresistant.

3.3.1.3 Epidermal Growth Factor and Radiation Sensitivity

Epidermal growth factor is a polypeptide hormone that induces the proliferation of epithelial cells after complexing with receptors on the cell's plasma envelope. Kwok and Sutherland (1989) examined the effect of this growth factor on a cell line derived from a human squamous carcinoma. Particularly when the factor was applied after irradiation, they observed a reduction in survival mainly because the shoulder width of the survival curve was decreased, i.e., because of a reduction in the cells' capacity for sublethal damage. Other properties of the cells that were examined were not changed, however.

3.3.2 Mutation

Mutations, which are defined as any heritable alteration in the composition of genomic DNA (see Moore et al., 1987; DeMarini et al., 1989), may be subdivided according to their size into "intragenic mutations" (changes within a single gene

including a base change at a single point), and "extragenic mutations" (larger changes involving deletion of the gene up to the loss of the entire chromosome; also called "multilocus" or "intergenic" changes). Chromosomal, extragenic mutations seem to be at least as important as intragenic mutations in cancer. As discussed in Task 2, specific chromosomal lesions are associated with a number of specific types of cancer, see Section 3.4.

A large number of methods have been used to detect genetic damage and to measure mutations. Test organisms have included bacteria, yeasts and molds, plants, fruit flies, cultured mammalian cells from a variety of species, laboratory animals, and even humans. In mammalian cells, quantitative data on the induction of mutations by radiation have been developed based on the detection of induced resistance to certain toxic chemicals. The techniques of molecular biology now permit analysis of the changes induced in the gene itself, or its product, including alterations in the base sequence of the gene.

Mutations in a particular gene generally have been studied as follows. Cells were treated with a mutagen that, among its other actions, may have killed a certain fraction of the population. The survivors were allowed to increase in number to allow for the expression of the mutation and to obtain a population large enough to measure the small proportion of cells that had lost the genetic marker of interest. This proportion was equated to the mutation frequency in the entire, original population. But because only those mutations in cells that remain viable could be scored, cells killed by lethal damage were not detected as mutants even though they may have suffered changes in the gene of interest.

Hence, when a marker gene is located on a monosomic chromosome containing information near the gene that is essential for viability, as is usually the case, damage involving regions flanking the gene can result in the inability to score that mutation because the cell is killed. As a consequence, in a monosomic chromosome multilocus mutations are frequently lost from analysis. The target size for cell killing by radiation is much larger than that of a number of constitutive genes in their natural backgrounds. Therefore, lethality may lead to an underestimation of the frequency of mutation per unit dose. For this reason, low-LET radiation appears to be a poor mutagen when the marker gene is on a monosomic chromosome--e.g., the X chromosome of rodent, hamster, or human cells--whereas, in fact, ionizing radiation can be shown to be an effective mutagen when account is taken of the full spectrum of possible heritable, genetic changes.

An advantage to studying mutagenesis of a gene in a monosomic chromosome, however, is that only one copy of the gene has to be changed. In contrast, when two functional alleles on a pair of homologous autosomes are present, in principle the net frequency would be the square of the frequency for mutating a single allele; that is, a net frequency that could be so low that it would not be detectable with the techniques that are available. As discussed below, other approaches have been developed for studying mutation rates of markers on autosomal chromosomes at frequencies that are well within the measurable range.

3.3.2.1 Point Mutations in Shuttle Vectors

In addition to studies of mutagenesis in the genomic DNA of cells, the techniques of recombinant DNA have been used to examine the molecular changes induced in specific DNA sequences called "shuttle vectors." In essential terms, a shuttle vector is a piece of DNA, a few hundred base pairs in length, that can

be introduced into a mammalian cell to form an epigenetic particle, or integrated into chromosomal DNA. Shuttle vectors have been engineered such that, after they have been subject to a mutagenic treatment, like radiation, they can be recovered from their host cell before or after the vector's damage has been influenced by the host's repair/misrepair machinery. The vector is then introduced into a bacterial host to permit the DNA of the vector to be amplified by selectively growing up the bacteria that have incorporated it. By this procedure, enough DNA can be synthesized to permit quantitative analysis of it via, for example, restriction-fragment-length polymorphism, and/or base sequence analysis. In general terms, the technique outlined above also can be used to analyze a sequence of DNA constitutive to a mammalian cell.

Because of the small sizes of shuttle vectors, they lend themselves to the scoring of localized changes like intragenic deletions/insertions and point mutations. Grossovsky *et al.* (1988) examined the mutations produced in the adenine phosphoribosyl transferase (*aprt*) gene of a Chinese hamster cell that was hemizygous for that gene (i.e., +/- for *aprt* because the gene was entirely absent from one autosomal chromosome). After exposure to 5.0 Gy of ¹³⁷Cs γ -rays, the frequency of mutations in this +/- cell was increased some 14-fold. The gene from surviving, mutated cells was recovered by incorporating it into an appropriate shuttle vector. Following transfection and amplification of the mutated gene in a suitable bacterium, it was subject to sequence analysis. Of the mutations recovered by this method, base substitutions occurred in 2/3rds with small deletions accounting for the remainder. A variety of base substitutions were recovered that were randomly distributed throughout the gene. The foregoing results were qualitatively consistent with earlier data by Grossovsky *et al.*, (1986) which indicated that most of the *aprt* mutants recovered by this method appeared normal when assayed by restriction-fragment-length polymorphism; that is, the intragenic changes could have been point mutations or deletions of up to 150-200 base pairs in length. Because of the limited size of the sequence of DNA that could be examined by the use of this method, and because only those mutants were recovered that retained at least part of the gene, the kinds of extragenic mutations that radiation could produce in mammalian cells at high frequency would not have been detected.

3.3.2.2 Intragenic and Larger Deletions

In a number of studies, the gene hypoxanthine-guanine phosphoribosyl transferase (*hgpert*), which is located on the X chromosome, has been used. The enzyme coded by this gene is able to incorporate 6-thioguanine, a toxic analog of guanine, into DNA. Consequently, only cells that have been mutated at the *hgpert* gene can survive exposure to 6-thioguanine following treatment with a mutagen. Because the *hgpert* gene is on the X chromosome, only one copy is present in a male cell or functions in a female diploid cell. Studies have also been conducted with the thymidine kinase (*tk*) gene. The *tk* gene is ordinarily present in two copies per diploid mammalian cell and codes for the enzyme that phosphorylates exogenous thymidine so that it can be incorporated into DNA. Through appropriate mutations and selection procedures, a line of human lymphoblastoid cells was developed (Liber and Thilly, 1982) that was heterozygous at the *tk* locus (i.e., +/-). Cells, in which mutants were induced in the single functioning allele, were selected for by growing them after irradiation in medium containing a toxic analog of thymidine, e.g., trifluorothymidine. When the latter compound is incorporated into DNA--that is, into the DNA of cells containing a functional *tk* gene--cells are killed so that only mutated cells survive to form colonies.

In contrast to ultraviolet light and certain chemicals like monofunctional alkylating agents, which produce primarily point mutations, mutations due to ionizing radiation frequently are found to involve larger genetic changes including deletions or translocations. Radiation-induced mutations, in the case of the *hgp*rt gene in Chinese hamster cells, included deletions of the portion of the X chromosome that contained the gene (Thacker, 1985); in the instance of the *tk* locus in human lymphoblastoid cells, the entire gene was frequently lost (Yandell et al., 1986; Liber et al., 1987).

The ability to detect radiation-induced chromosomal mutations frequently has been limited by the proximity to the gene of interest of essential genetic information. This limitation has been relieved somewhat by the development of assay systems such as those based upon the use of the mouse cell line L5178Y, in which the *tk* gene is heterozygous +/-, and the Chinese hamster cell line transfected with a bacterial gene that codes for xanthine-guanine phosphoribosyl transferase (see DeMarini et al., 1989). In both of the latter assay systems, radiation-induced frequencies were ~10 times greater, per surviving cell/Gy, than had been observed for the *hgp*rt gene on the X chromosome especially in the low-dose region. An assay system at least as sensitive as the preceding two is one based upon the use of a hybrid cell, designated A_L, in which a human chromosome 11 is contained in the genetic background of a Chinese hamster cell. Developed by Waldren et al., (1979, 1986), the radiation-induced frequencies of mutation were some 100-fold greater than they were with the *hgp*rt gene presumably because the markers in question reside in the human chromosome most of which is not required for viability. Thus, A_L cells have proven to be well suited to measurement of mutation frequencies in the low-dose regions of various mutagens including radiation (Puck, 1985; Waldren et al., 1986). Mathematical relationships between killing and mutation have been developed for this assay (Parker et al., 1988).

3.3.2.3 Frequencies of Induction, Hemizygous Genes

Although genetic markers, such as the *hgp*rt gene on an essential monosomic chromosome, may provide reasonably accurate and convenient measures of mutation for agents that produce mainly point and other intragenic changes, such as ultraviolet light, in such assays chromosomal mutations, including nondisjunctional changes, can be appreciably underestimated. Deletions at the *hgp*rt locus larger than ~40 kb are not observed, presumably because they would extend into vital genes and are selected against by cell death. Yet, data from a variety of human cancers have shown that precancerous, but functionally normal cells, may be viable even when they contain deletions as large 1000 kb when only one allele of a chromosomal pair is affected. But a mutation in the second allele can lead to cancer (see Section 3.4).

An indication that human cells can remain viable even after suffering large chromosomal deletions comes from cytogenetic studies on the relationship between cell killing and microscopically visible, acentric chromosomal fragments (Cornforth and Bedford, 1983). A one-to-one correlation was found between the proportion of cells killed and the proportion of cells containing at least one acentric fragment. From these results, the inference may be made that fragments smaller than those visible in the light microscope were not lethal. An upper limit of the size of such submicroscopic fragments could be ~5000 Kbp, that is, large enough to result in the loss of several to 10 genes (Ur̄laub et al., 1986).

3.3.3 Neoplastic Transformation

Although significant progress has been made in reference to oncogenes and neoplastic transformation in general, the data are quite limited in the context of radiation induction of this endpoint. It is likely that part of the motivation for the studies on oncogenes and radiation lethality has been the assumption that they would have a bearing on the mechanism of radiation-induced transformation. However, thus far the data available do not suggest a strong coupling between the two endpoints (see Subsection 3.2.1).

3.3.3.1 Rodent Cells

Borek et al., (1987) transfected normal 3T3, 10T1/2, and rat-2 cells with DNA extracted from transformed colonies of 10T1/2 cells. They found that DNA from unirradiated cells or from irradiated, nontransformed cells did not produce transformants in any of the recipient cultures, but recipient cells were transformed by transfecting with DNA from transformed cells. Transfectants produced tumors in immunologically deficient mice. By the use of appropriate restriction endonucleases (enzymes that produce scissions in DNA at specific 4 to 8 base-pair palindromic sequences) and applying appropriate DNA probes, a number of oncogenes could be eliminated as those responsible for the transformed phenotype. Among the oncogenes that were ruled out were the three kinds of *ras* oncogenes; *Ki-ras*, *Ha-ras*, and *N-ras*.

Although limited in quantity, some data are becoming available regarding the changes in gene products that are associated with radiation and transformation. After irradiation, normal or transformed cultured cells had altered patterns of proteins whose expression, in some instances, was correlated with sensitivity to cell killing (Lambert and Borek, 1988). This approach may be a fruitful in future studies.

3.3.3.2 Human Cells

Although dose dependencies are not available of the induction of transformation with normal human cells, studies have been performed with cells derived from a human cervical carcinoma, HeLa cells, fused to form a hybrid with a normal human skin fibroblast. These hybrid cells can be transformed by radiation as indicated by the expression of a tumor-associated surface antigen (Redpath et al., 1987; Sun et al., 1988). Further, the ability of these cells to produce tumors in immunologically suppressed mice has been shown to be due to the loss of information contained on the chromosome 11 derived from the normal human cell (Saxon et al., 1986). As opposed to the dominant action made evident when genomic DNA from radiation-transformed rodent cells was used to transfect 3T3 cells (Borek et al., 1987), the transformed phenotype of the hybrid cells above appears to be due to a radiation-induced loss of suppressor gene action from chromosome 11.

3.3.4 Tumorigenesis in Rodents

The demonstration of an association between certain oncogenes and chemically-induced tumorigenesis led, quite naturally, to similar studies with radiation.

3.3.4.1 Skin Tumors

Using electron irradiation to limit the depth of exposure of the skin, tumors were induced in rats the genomic DNA from which was analyzed for the expression of specific oncogenes via the transfection/transformation assay of NIH 3T3 cells in culture (Sawey et al., 1987). The DNA from 6 of 12 tumors was found to transfect an activated Ki-ras oncogene, and 10 of the 12 tumors also exhibited amplification of the c-myc oncogene. Three clear-cell carcinomas contained activated c-myc and Ki-ras oncogenes. These results support the evidence from studies of chemically-induced neoplasia that the *ras* and *myc* oncogenes may be involved in a cause/effect relationship in tumor induction (Balmain and Pragnell, 1983; Land et al., 1983; Barbacid, 1986).

3.3.4.2 Thymic Lymphoma

The association of activated or mutated *ras* oncogenes with chemically-induced tumors led to an investigation of their involvement in radiation-induced thymoma in mice. Using a dose fractionation protocol, which induces lymphomas with high probability, followed by the transfection assay of NIH 3T3 cells as already described, Pellicer and his associates demonstrated: 1) that the genomic DNA of the thymomas could transform 3T3 cells and that the transformed cells contained extra copies of the Ki-ras oncogene (Guerrero et al., 1984a); 2) that DNA from the transformed 3T3 cells contained a mutation in the first exon (i.e., the first coding unit) of the *ras* oncogene (Guerrero et al., 1984b); 3) that the mutations in Ki-ras genes from 3T3 cells were in the 12th codon (Diamond et al., 1988); 4) that similar mutations were also present in the thymomas whose DNA transformed 3T3 cells (Guerrero et al., 1984b); and 5) that only 5 out of 12 radiation-induced thymomas did not contain *ras*-transforming activity (Newcomb et al., 1988). Thus, these studies, along with those of tumor formation in skin, suggested a significant involvement of the *ras* oncogene in radiation-induced neoplasia. Alternatively, because not all tumors appeared to involve an activated *ras* oncogene, it is possible that the coupling between that gene and tumorigenesis is circumstantial and not causal.

3.3.4.3 Acute Myeloid Leukemia

The molecular biology of radiation-induced, acute myeloid leukemia in mice has been under study by Cox and his associates (Silver et al., 1990). In several strains of mice, cells of this leukemia contain deletions and/or rearrangements in chromosome 2 (see Breckon et al., 1988). To trace the time of appearance of the aberrations in chromosome 2 that might be connected with the neoplasm, marrow cells from male mice were exposed in suspension to 3 Gy of X-rays and then transplanted into female recipients whose marrow had been ablated. The Y chromosome was used as an indication that a graft of male cells had taken place in female recipients. In this way, in only 5 days after the transplantation, rearrangements in chromosome 2 were identified as a consequence of the irradiation. Some of the transplants yielded clones that proliferated well, but most declined in time. None indicated progression toward acute myeloid leukemia. These observations led to the conclusion that changes in chromosome 2 resulting from irradiation constitute a broad class of genomic changes only a subset of which are able to develop into leukemia (Silver et al., 1987).

Cytogenetic and molecular analyses ruled out an involvement of certain oncogenes that are located on chromosome 2. Because the interleukin cytokine genes had been mapped to the region of chromosome 2 where Cox and collaborators

had identified frequent changes, they examined the interleukin gene *IL-1 β* and found changes in its pattern of methylation. The latter observation suggested that deregulation of *IL-1B* could be causally associated with leukemia.

3.3.5 Gaps in Knowledge, the Role of Genetic Changes

The information reviewed in this section on radiation-induced endpoints is not extensive. Hence, an overall need for more data on the roles of genes and oncogenes in radiation endpoints is suggested. In addition, certain specific issues require further examination.

3.3.5.1 Oncogenes in Cell Killing

The fact that the effective target sizes for the production of various kinds of lesions in DNA are large compared to that for lethality implies that appreciable repair of DNA damage occurs in repair-competent cells (Elkind et al., 1987). An observation that is consistent with the foregoing is the finding that repair-defective mutants have been identified that are hypersensitive to radiation killing (Stamato et al., 1981). In view of the relatively minor changes in survival responses attributed to the oncogenes discussed in Subsection 3.3.1, it is unlikely that they are repair genes. A more likely possibility is that, by modulating the proliferative status of cells, oncogenes influenced the degree of damage expressed as a secondary factor in survival response. Still, it is relevant to determine if and how altered oncogene expression influences cell killing.

3.3.5.2 Point Mutations and Intragenic vs. Large Chromosomal and Extragenic Changes

The data indicate that ionizing radiation can cause point mutations, other intragenic changes, and larger changes such as gene deletions ranging up to the loss of an entire chromosome. To relate somatic mutation to the heritable alterations that underlie neoplastic transformation, however, requires: that the probabilities of these various kinds of events should be known; and that the influence of these changes on the growth potential of the cell should be determined. A given somatic mutation might occur with high probability but if it confers a growth disadvantage on the cell, it may not contribute significantly to neoplastic growth. Alternatively, a mutation that confers a growth advantage could be important even if it is induced by radiation at a modest or low frequency.

The evidence indicates that point mutations in the *ras* oncogene can act dominantly. Further, it is likely that any genetic change from a point mutation to the loss of a chromosome--as a result of nondisjunction, for example--could result in the loss of function of an anti-oncogene/suppressor gene. Hence, it is important to know which of these two mechanisms of transforming cells plays the more important role. Connected with the foregoing is the likelihood that deletions and larger chromosomal changes, which involve genomic information required for viability, will be discriminated against. Discrimination also might reflect the differential repair/misrepair of various types of lesions in DNA (e.g., Stamato et al., 1981). As illustrated in Section 3.4, several human cancers have large extragenic mutations, which would appear to favor such changes over intragenic alterations in neoplasia.

Although the evidence supports the hypothesis that radiation is more likely to effect neoplastic transformation via chromosomal changes as opposed to intragenic changes including point mutations, either mechanism could initiate the transformation process whether or not the transformed phenotype results from a dominantly acting oncogene, or an anti-oncogene one or both of whose alleles is affected. Further, it is not known to what degree small intragenic changes vs. chromosomal changes are responsible for the transformation of fibroblasts from different species, or the cells from different tissues of the same species including, particularly, epithelial cells.

3.3.5.3 Oncogenes in Neoplastic Transformation

The evidence from studies with chemical carcinogens has associated certain oncogenes with the phenotypic changes of transformation. Which oncogenes are responsible for radiation-induced transformation is not known. When the latter genes are identified, their mode of action would have to be worked out; for example, dominant action as in the work of Borek *et al.*, (1987) with 10T1/2 cells, or suppressor action as suggested by the results of Saxon *et al.* (1986) relative to the radiation study with HeLa cells of Sun *et al.*, (1988). Furthermore, because of the evidence that two or more oncogenic changes may be required in tumorigenesis (e.g., Land *et al.*, 1983), it would be important to determine how many oncogenes/anti-oncogenes are, or can be, responsible for particular types of transformation. The relative simplicity of cell culture systems (or even *in vitro/in vivo* systems) compared to those based only on tumor induction, suggest that the question of the number of activated oncogenes initially should be examined with cells *in vitro*.

3.3.5.4 Oncogenes in Multistep, Radiation Tumorigenesis

The demonstration that certain inducing agents other than ionizing radiation can cause neoplasia via a series of steps identified operationally as *initiation*, *promotion*, and *progression* suggests that similar steps should be involved in radiation tumorigenesis. Hence, in addition to identifying which and how many oncogenes may be involved, and in what way they become activated, it is necessary to know what roles they play in the overall process. For example, the results of Guerrero *et al.*, (1984a,b) suggest that the activation of a *ras* oncogene by a point mutation is responsible for the initiation of the thymomas that were induced by radiation. What is not clear, however, is whether the mutated *ras* genes were a cause or an effect of the neoplastic process. Similar considerations apply to the involvement of both the *ras* and *myc* genes in the studies of radiation-induced skin cancer (Sawey *et al.*, 1987), and the activation of the *11-1 β* gene in myeloid leukemia (Silver *et al.*, 1990). With respect to progression, the activation of the gene coding for the protease transin has been proposed as a factor responsible for the conversion of skin papillomas to carcinomas (Matrisian *et al.*, 1986).

3.4 ONCOGENES IN HUMAN NEOPLASIA

As reviewed in Task 2, recent advances in our understanding of the roles of oncogenes and anti-oncogenes (tumor suppressor genes) in the control of cell proliferation have identified several mechanisms that can result in the neoplastic state. In Subsection 2.3.2, several kinds of altered oncogenic properties were described, and in Section 2.5 the role of dominant as opposed to recessive gene action was discussed. Genetic changes ranging from point

mutations to chromosome loss can be involved in spontaneous tumors, and in Section 3.3 similar oncogenic changes in radiation-induced neoplasia were identified. An inappropriate or excessive expression of an oncogene product, or a lost ability to express an anti-oncogene product, due to intragenic or extragenic changes including chromosomal rearrangements or deletions, often appear to be critical determinants of the neoplastic state.

A widely acknowledged characterization of oncogenesis is that it is the result of the multiple steps of *initiation*, *promotion*, and *progression* as in Fig. 2.7. It is becoming clear, however, that in general a more detailed mechanistic description is required. Further, as the discussion in this Section will illustrate, it now seems likely that the multiplicity of steps may vary widely depending on the type of neoplasm.

In the material to follow, examples are given of the roles of certain oncogenes in neoplasia and, where they are known, some of the steps are described that appear to be involved. In regard to the number of steps, two extremes will be discussed. Relatively few steps have been identified in human leukemia whereas many changes are associated with colon or lung carcinoma. It is important to note that the incidence of different cancers is not necessarily related to the number of steps presumably because of differences in the etiologies of the neoplasms and in the probabilities of the sequential changes. That is, the probability of the occurrence of a given step may be conditioned, or made more likely, by the occurrence of one or more earlier steps. Still, the number of steps may influence the time to the appearance of a particular tumor. Leukemias, which appear to involve relatively few steps, often develop earlier in life--or sooner after exposure to radiation or other oncogenic agents--than, say, colon cancers which appear later in life and have longer latency periods after exposure to radiation.

3.4.1 Burkitt's Lymphoma and c-myc

In Subsection 2.3.2.2, the three specific chromosomal translocations, one or another of which is present in 100 percent of Burkitt's lymphomas, were described. Also described was the close proximity in which the autosomal oncogene c-myc was found relative to the active promoter of the immunoglobulin genes as a result of these chromosomal translocations. In these tumors, the translocated c-myc allele is actively transcribed whereas the allele that was not translocated remains controlled and is not transcribed (Hayday et al., 1984). Burkitt's lymphoma is an example where an increase in the quantity of the product of an oncogene, c-myc, is associated with the neoplasm which suggests a cause/effect relationship.

There is some evidence that another "event," in addition to a chromosomal translocation, may be involved in the development of Burkitt's lymphoma. Apparently, either the translocation of c-myc or the infection with the Epstein-Barr virus (EBV) can cause immortalization of B-type, white-blood cells but neither change by itself can result in the full development of the lymphoma. Cells immortalized with EBV, however, can undergo full malignant transformation upon transfection with c-myc (see Klein, 1987). Further, activation of c-myc by itself in transgenic mice does not induce malignancy (Leder et al., 1988). To produce a neoplasm, c-myc activation appears to be necessary but not sufficient. The foregoing suggests the scheme shown in Fig. 3.4 as the *growth-state diagram* for the development of Burkitt's lymphoma. (See Section 3.5.2 for further discussion of the term *growth-state diagram*.) Although the evidence is lacking

Fig. 3.4 Schematic representation of the growth states, and the cellular/chromosomal changes involved in the induction of Burkitt's lymphoma. The designation " $t(8;14)(q24;q11)$ " describes a reciprocal exchange, of the long arm (q) of chromosome 8, starting from band 24, with the long arm (q) of chromosome 14 starting at band 11. Other exchanges are designated similarly.

that Burkitt's lymphoma is radiogenic, the facility with which ionizing radiation can produce chromosomal rearrangements, as in this neoplasm, suggests that radiation could be effective in inducing neoplasms with similar chromosomal translocations.

The importance of gross chromosomal changes has become recognized and appreciated only in the past 10-15 years. In this period, new techniques for characterizing human chromosomes were developed that permitted whole, or sections, of chromosomes to be identified (see Figs. 2.2 and 2.3) and therefore losses or rearrangements of chromosomes in tumors to be detected. Thus, Yunis et al., (1981) and LeBeau and Rowley (1986) were able to identify deletions in human leukemic cells, and Yunis et al. (1981) showed that the 50 percent of leukemias in which there were no "cytogenetically detectable" chromosomal changes microscopically identifiable by the older methods actually had abnormalities in 24 of 24 cases when new high-resolution banding techniques were applied.

3.4.2 Chronic Myeloid Leukemia and c-abl

A chromosomal change in human, chronic myeloid leukemia appears to involve a translocation between chromosomes 9 and 22 described as t(9;22)(q34;q11); that is, the terminal portion of chromosome 9 starting at position 34 of its long arm (the "q" arm) is exchanged with the terminal portion of chromosome 22 starting at position q11. This exchange generates the so-called Philadelphia chromosome (Ph¹), which occurs in some 85-100 percent of these tumors (LeBeau and Rowley, 1986). The break-point on chromosome 9 is very near the c-abl oncogene; in the translocation, this gene is moved to a critical point on chromosome 22 known as the "break-point cluster region," bcr. The translocated c-abl oncogene produces a larger-than-normal mRNA transcript containing both abl and bcr sequences, which is translated into an abnormal protein, a tyrosine kinase, having an increased and abnormal ability to phosphorylate tyrosine residues (Shtivelman et al., 1985). Thus, in contrast to Burkitt's lymphoma, where a quantitative alteration of an oncogene (c-myc) product is involved, in chronic myeloid leukemia it is likely that a qualitative alteration in an oncogene product occurs.

More than one step appears to be required in the transition of chronic myeloid leukemia from the chronic phase to what is known as the "blast crisis," or the terminal acute phase. Here, as many as 80 percent of the neoplasms show chromosomal changes, in addition to the production of the Ph¹ chromosome, that often precede the appearance of the clinical symptoms associated with the blast phase. Frequent chromosomal changes that are associated with this transition include the presence of an additional chromosome 8 or 19, a second Ph¹, or an isochromosome consisting of two long arms of chromosome 17 joined at their centromere, designated i(17q). Combinations of these changes also occur.

Chronic myeloid leukemia, which is radiogenic, may be characterized by the growth-state diagram in Fig. 3.5. A property of ionizing radiation that would be particularly relevant would be the facility with which it breaks chromosomes.

3.4.3 Solid Tumors

Although less well defined for solid tumors than for leukemias, the details of growth-state diagrams are emerging for some solid tumors. It is becoming apparent that the development of a tumor involves a number of specific mutations including deletions of parts of, or entire, chromosomes. In some instances, there is loss of heterozygosity from which it may be inferred that recessive-

Fig. 3.5 Schematic representation of the growth states, and the cellular/chromosomal changes involved, in the expression of human myeloid leukemia. Designation of chromosomal exchanges as in Fig. 3.4.

suppressor-gene action plays a role in the initiation of unregulated growth and/or the further steps of promotion/progression that lead to neoplasia (see Ponder, 1988; Glaser et al., 1989; Sager, 1989).

The two-hit model of Knudson (1978), based on recessive-, suppressor-gene action, has been demonstrated most clearly for retinoblastoma, a rare cancer of the eye. For this neoplasm to develop, two allelic mutations are required that inactivate both autosomal copies of a single gene, the *RBI* gene. In familial retinoblastoma, one of the two mutations is inherited through the germ line. At a cellular level, each allele acts dominantly because one nonmutated gene is sufficient to maintain normalcy (see Fig. 2.5). Cytogenetically visible chromosomal deletions have been identified in many retinoblastomas on the long arm of chromosome 13. Through the application of the techniques of molecular biology, the *RBI* gene has been cloned, and the protein corresponding to the gene has been identified although its function remains to be worked out.

In other solid tumors, evidence of more complex mutations has been accumulating as illustrated by the examples to follow.

3.4.3.1 Colorectal Carcinoma

At the present, one of the most completely specified and complex growth-state diagrams of a solid tumor is that of colorectal cancer (Fearon, et al., 1990; Feinberg et al., 1989; Vogelstein et al., 1989). A growth-state diagram, after Vogelstein et al. (1989), is shown in Fig. 3.6.

Frequently, these tumors appear to start with increased proliferation (hyperplasia) that precedes the appearance of abnormal, noncancerous growths (adenomas) some of which may go on to become carcinomas. Also sketched in Fig. 3.6 is the involvement of a familial condition that can lead directly to the adenomatous growth state. Families in which the condition *familial polyposis coli* is expressed can have offspring that, at an early age, develop hundreds to thousands of benign colonic polyps, which are virtually always precursors of colon carcinomas. It has been found that many of these polyps have associated with them a deletion in the long arm of chromosome 5 (White, 1989). Other evidence indicates that the DNA of adenomas--identified in Fig. 3.6 as starting with adenoma I--is deficient in its content of methyl groups. Because genes that are methylated tend to be inactive, the condition of hypomethylation suggests more active transcription of such genes in adenomas than in the normal cells of the colon.

Further genetic changes that have been demonstrated in colon carcinoma include mutations in the *Ki-ras* gene and allelic losses in chromosomes 17 and 18. Operationally, these changes may mean that two additional stages of adenomatous growth occur before a carcinoma is expressed (Vogelstein et al., 1989). Losses of alleles in chromosomes 17 and 18 also have been associated with the advanced malignancy of these tumors. All told, the evidence suggests that at least five mutations are required to give rise to a carcinoma although it is not known whether they have to appear in the particular sequence, *deletion/mutation on chromosome 5 → DNA hypomethylation → ras mutation → deletion on chromosome 18 → deletion on chromosome 17*.

Fig. 3.6 Growth states, and oncogenic/chromosomal changes, in human colorectal carcinoma. (Adapted from Vogelstein et al., 1989.)

3.4.3.2 Mammary Carcinoma

A complex set of genetic changes have also been associated with human breast cancer. These are illustrated schematically in Fig. 3.7. Because the sequence of events is not well understood, the construction of a growth-state diagram has not been attempted. In broad terms, a number of oncogenes have been found to be amplified, suggestive of dominantly-acting genetic changes, and loss of heterozygosity has been detected on chromosomes 1q, 3p, 11p, 13q, and 17p (Callahan and Campbell, 1989). In addition, it is noteworthy that a number of the changes indicated in Fig. 3.7, such as the loss of the *RBI* gene located on chromosome 13, are also associated with other neoplasms as well.

3.4.3.3 Lung Carcinoma

As is evident in Fig. 3.8, the situation for lung carcinoma is similar to mammary carcinoma in its complexity. In lung carcinoma, as well as in mammary carcinoma, *17-2* is altered and an allelic loss(es) has been identified on chromosome 3p (Wang-Peng, 1989). Just which and how many of the changes indicated in Fig. 3.8 are required for the neoplastic phenotype to be expressed, and which and how many reflect various degrees of promotion/progression to different states of malignancy, is not known (Birrer and Minna, 1989; Weston et al., 1989; Takahashi et al., 1989).

3.4.3.4 Other Solid Tumors

Besides the examples of colorectal, mammary, and lung carcinomas, the multiple-hit, suppressor-gene model of oncogenesis is strongly implicated in Wilms' tumor, rhabdosarcoma, insulinoma, acoustic neuroma, renal cell carcinoma, neurofibrosarcoma, hepatocellular cancer, and bladder cancer. Large-scale chromosomal rearrangements, of the kinds that can be produced by ionizing radiation, have been identified in the karyotypes of these neoplasms. The large number of mutations implied by the changes in these tumors suggests that some of them may be a consequence of, and dependent upon, others. Which are which is not known.

3.4.4 Gaps in Knowledge of Tumorigenesis

The progress in understanding tumorigenesis at the molecular level that is summarized by Figs. 3.6-3.8 is impressive. Still, in large part, these diagrams constitute molecular/genetic taxonomy. It is quite likely that these diagrams include the altered genetic states that are responsible for unregulated growth. Still, what the growth-state diagrams do not make clear is how and why these changes occur. To significant degrees, therefore, the information in these figures constitutes descriptions and not explanations.

With the essential assumption that a full understanding of the mechanism of neoplasia requires that *cause* and *effect* should be understood, the following are identified as gaps in our current knowledge.

1. What are the products of oncogenes and antioncogenes and how do they function in normal cells? How is the expression of these products normally regulated, and how is this regulation altered by mutation or other events, such as, changes in the availability of growth factors, etc.? How do the kinds of mutations generated by radiation cause loss of growth control?

Fig. 3.7 The oncogenic/chromosomal changes that have been associated with human mammary carcinoma. The growth states that are associated with these changes have not been identified as yet.

Fig. 3.8 The oncogenic/chromosomal changes that have been associated with human lung carcinoma. The growth states that are associated with these changes have not been identified as yet.

2. In normal cells, control of gene expression remains intact as long as mutations are not expressed. What are the roles of repair/misrepair mechanisms, especially those involved in damage from ionizing radiation, in maintaining normal or abnormal gene expression?
3. What are the roles of different kinds of mutagenic events--point mutations, recombinations, gene conversions, aneuploidy, amplifications, translocations, deletions, and so on--in effecting the transitions between growth-states? With what relative frequencies are these changes produced by radiation of different qualities, delivered at high and at low dose rates?
4. Is ionizing radiation effective mainly in *initiation* of the neoplastic process or does it also contribute to *promotion* and/or *progression*?
5. What is the connection between the histology of a tissue and the genetic changes that lead to the transformed state? Which changes are *necessary* and which are *sufficient* to induce a given growth state (see Subsection 3.5.3.4)? Which changes *cause* and which are the *result* of the altered growth states? Is the accumulation rather than the order of the changes important? Are the changes quantal--that is, are growth controls turned on or off--or altered in some graded way?
6. With reference to colorectal cancer (Fig. 3.6), is the order of the growth states unique? What growth states apply to other neoplasms like mammary and lung carcinomas (Figs. 3.7 and 3.8), and what are their genetic characterizations?
7. Is hyperplasia a concomitant of abnormal tissue histology? Are genetic changes required for hyperplasia?
8. In growth-state transitions, what is the role of immortalization, where does it apply, and is it connected with aneuploidy? What genes regulate immortalization and how do they function (Sugawara et al., 1990). What influence does aneuploidy have on the evolution of a tumor? Is aneuploidy a *cause* or *effect*?
9. What is the relative importance of dominant vs. recessive oncogene action?
10. Can neoplasms result from the activation of single oncogenes, i.e., from the dominant action of a single gene? If so, do only certain oncogenes have this ability? Is the activation of two or more oncogenes required; are there generalizations with respect to which and how many? Is "dosage" of oncogenic activation important; that is, the degree of activation and the number of oncogenes so activated? Does dosage of activation have a bearing on the latency period?
11. Can neoplasms, in addition to retinoblastoma, result from the inactivation or loss of autosomal alleles i.e., only from recessive gene action? Can the loss of more than one set of alleles give rise to the same neoplasm (e.g., Wilms' tumor; Sager, 1989)?
12. Can changes in specific oncogenes be associated with the operational steps of *initiation*, *promotion*, and *progression*?

3.5 RADIATION TUMORIGENESIS, FEASIBILITY OF REDUCING UNCERTAINTIES

It is generally acknowledged that it is not possible to arrive at accurate dose dependencies for radiation-induced human neoplasia from epidemiological studies alone, particularly when all of the relevant parameters are to be accounted for, and especially at low doses and low dose rates. In addition to the expectation that frequencies would be low for doses to which the population at large is exposed--for example, 0-10 cSv--it is not feasible to determine from epidemiological data how frequencies of the induction of cancer depend upon: the type of tumor and tissue of origin; the quality of the radiation; the rate of exposure; and the influence of possible confounding variables such as smoking, exposure to asbestos, ultraviolet light, and various chemicals including those that are known to be promoters/antipromoters of carcinogenesis (see BEIR V Report, 1990). Additional epidemiological complications could reflect the genetic variability among individuals relative to cellular repair capabilities, immunological competencies, and the functional activity of anti-oncogenes. In view of these imponderables, it is unlikely that the current state of knowledge will lead to significant reductions in the uncertainties of the risks of radiation-induced cancer from what are indicated in the BEIR V Report (1990).

The question that should be addressed, therefore, is: Is it feasible to reduce the uncertainties of radiation-induced neoplasms from an expanded knowledge of the underlying mechanism(s) of the induction of unregulated cellular proliferation *in vivo*? The answer to be developed in this section is a qualified "yes." The dramatic developments that are occurring in the oncogenic descriptions of neoplastic growth states offer promise that in the next several years the cellular/molecular biology of human cancer will become understood in general terms, and that of certain neoplasms in specific terms (see Section 3.4). Consequently, it is likely that the oncogenic basis of a number of spontaneous human cancers will soon be known.

In addition to the requirement that the foregoing should become a reality, to make the radiation problem tractable it is also necessary that the following major objectives should be accomplished:

- 1) the radiation-induced genetic changes have to be identified that are responsible for the neoplastic transformation of cells *in vitro* and in animal tissues using methods that are currently available or new methods as may be required;
- 2) a rational framework is needed to relate radiation-induced transitions between growth states in experimental systems to the transitions between growth states of human neoplasms; and
- 3) strategies are needed to enable animal data on radiation tumor induction to be extrapolated to people in a reliable way.

3.5.1 Radiation-Induced vs. Spontaneous Neoplasia

As noted in reference to Fig. 2.1, inherited genetic anomalies--like those associated with repair deficiencies, e.g., xeroderma pigmentosum and ataxia telangiectasia--can play an important role in susceptibility to cancer. In addition, in familial cancer a recessive mutation may be inherited through the germ line that can predispose to cancer susceptibility (see Section 3.4). Factors, such as immune competence, are known to be important in cancer

susceptibility. The role of somatic mutations in unregulated growth also has been well established.

A somatic mutation can result from genetic changes ranging from a single base change in DNA to a gross chromosomal rearrangement. If the genetic change does not kill the cell, the mutation may be expressed as an alteration in a growth property of the cell and thus contribute to its unregulated growth. It is also known that the mutation itself may not directly affect cellular growth potential. For example, if the mutation leads to a change in the fidelity with which DNA is replicated, or in the effectiveness of DNA repair mechanisms, or in the normal processes of differentiation, the progeny of the mutated cell may have a selective growth advantage and, hence, initiate neoplastic growth (see Fig. 2.7). However, in each of the foregoing examples, the mutation would not necessarily have been in the gene that was ultimately connected with the subsequent deregulation of growth.

Although the basis for the appearance of spontaneous cancers is not fully understood, it is likely that a large fraction of them is caused by mutagenic/carcinogenic environmental and dietary agents. Radiation constitutes only one of many potentially causative agents; various estimates attribute 3-5 percent of the spontaneous incidence to ionizing radiation in the environment. Intragenic as well as large extragenic genetic changes can be caused by chemicals as well as by radiation. However, as noted in Subsection 3.3.2, it is likely that radiation differs qualitatively from other inducers in that inherently it is more capable of producing large genetic changes. The average energy deposited per absorption event by a low-LET radiation, ~60 eV, is large enough to break any molecular bond and, in addition, clusters of ionizations can be produced whose dimensions are large enough to rupture a number of bonds in close proximity due to direct action, or to direct plus indirect action due to the diffusion of reactive species, like OH⁻ radicals, to the site. Other agents also produce extragenic changes but, in general, the number produced per lesion in DNA is appreciably greater for ionizing radiation.

Thus, in contrast to chemical mutagens and most other genotoxic agents, at equal degrees of killing, radiation can be expected to produce disproportionately more chromosomal rearrangements. Many of these may result in configurations that are not consistent with viability. Nonetheless, because of the large number of cells at risk and the large proportion of genetic events per unit dose, it is possible that radiation-induced neoplasia is initiated by somatic mutations the spectrum of which is qualitatively different from those due to other agents. The efficiency with which genomic lesions are registered by radiation may be illustrated as follows. In a single diploid mammalian cell, which would contain ~1 x 10⁻¹¹ gm of DNA, each cGy of a low-LET radiation would produce ~10 single-strand lesions and 1-3 double-strand scissions in the DNA. In addition, 1-2 crosslinks between DNA and proteins are produced per cGy (Ramakrishnan et al., 1987).

Two related arguments suggest that it is logical to base a study of radiation-induced cancer on the genetic changes found in spontaneous human neoplasms. Whatever may be the causative steps for the induction of human cancers, small, protracted doses of a low-LET radiation can be expected to yield small increases in cancer frequencies with the likelihood that the molecular changes responsible for these increases will be qualitatively similar to those which give rise to spontaneous neoplasms. This is the first argument for

modelling a radiation study on the kinds of genetic/molecular changes that characterize spontaneous human neoplasms.

The second argument is connected with the nature of the somatic mutations that are likely to be produced by low doses of a low-LET radiation delivered during a protracted exposure. In general, the growth-state diagrams in Figs. 3.4-3.8 indicate that both intragenic and extragenic changes are involved. Extragenic, multilocus mutations require chromosomal rearrangements and/or deletions which, in turn, usually result from two or more scissions of the two-stranded DNA backbone of a chromosome. (Note, although the DNA in a single chromosome in the nucleus of an animal cell is compacted to a length of one to a few micrometers, genetic and structural evidence indicate that a chromosome contains a single, linear molecule of DNA whose length can be $1-5 \times 10^4$ times longer.) Chromosomal multiple-hit events, precursors of chromosomal rearrangements, may result from the traversal of a single ionizing particle; however, with increasing dose, they occur with increasingly greater probability from two or more traversals. Hence, at a high dose rate, it is likely that a proportion of large genetic mutations will be induced with the square and higher powers of the dose. Cell lethality is also likely to increase with the same qualitative dependence on dose because many of the residual chromosomal changes are not compatible with viability. In the region of low dose, and particularly when exposures are protracted, lethality will be minimal because the number of traversals will be fewer, and because the damage due to individual traversals may be repaired before damage from additional traversals is registered. For these reasons, with decreasing dose and with increasing duration of exposure, it is likely that the ratio of radiation-induced large extragenic to intragenic mutations would decrease such that a spectrum of mutations closer to those found in spontaneous neoplasms would result.

Although the first argument--an extrapolation from background to small but higher doses--is similar to the second--an extrapolation to background from higher doses--the former differs from the latter in that it does not invoke a qualitative shift in the spectrum of mutations. That such a shift should occur is a reasonable, but an unproven, supposition and, therefore, constitutes a gap in our knowledge.

3.5.2 Growth States in Human Disease

Progress in the understanding of the normal roles of proto-oncogenes has facilitated the identification of the molecular changes associated with unregulated growth and/or abnormal differentiation. Examples of several of the most fully characterized human neoplasms were discussed in Section 3.4. Specific changes in oncogenes have been associated reasonably well with each of the neoplasms that were described. *Cause and effect* is implied in the growth-state diagrams that were discussed but has not been clearly established. Additional gaps in the roles of the genetic changes that have been discovered were noted in Subsection 3.4.4. A common feature of the genetic descriptions of the neoplasms discussed in Section 3.4--as in Fig. 3.6--is the sequential, and usually progressive, nature of the disease.

Thus, the transitions from a normal progenitor cell to cells that are hyperplastic (amplified in number) and/or abnormally differentiated, have associated with them heritable phenotypes that are characteristic of the growth states in question. For any particular neoplasm, it has not been established that unique pathways exist for the progressive transition through its growth

states. Furthermore, it is not as yet clear if, and to what degree, overlap exists among the genetic changes that characterize a given growth state of a particular disease.

In spite of the foregoing uncertainties, it is reasonable to assume that the genetic characterization of a histologically identifiable cellular state--for example, as in colorectal carcinoma, Fig. 3.6--is the result of one or more discrete, presumably sequential mutations. Thus, the research that has led to the genetic/molecular taxonomies of particular human cancers suggests that a growth-state diagram may be a valid way of specifying the pathway for the development of the disease. Although uniqueness of these pathways is not clear, provisionally, uniqueness can be assumed. Also, a knowledge of a pathway is not the same as knowing how the transitions from state-to-state--that is, the genetic changes--are produced.

3.5.3 Radiation-Induced Oncogenic Changes

The growth-state diagrams in Figs. 3.6-3.8 illustrate the kinds of oncogenic changes in the genomes of human cells and their progeny that are associated with developing neoplasms. Broadly stated, these consist of activated or inactivated *cancer genes*; that is, oncogenes that are activated and act dominantly, and anti-oncogene/suppressor genes that are inactivated. There is evidence that anti-oncogenes may exist that act dominantly by exerting negative control on the expression of an allelic oncogene thus maintaining the normal state of growth. To develop information on the efficacy with which radiation can give rise to these changes, at the outset it is necessary to know the dependence of their induction on physical and biological parameters. Among the physical parameters are the radiobiological dependencies on dose, dose rate, dose protraction (i.e., brief exposures spread over time periods of the order of, and longer than, cell-cycle times), and LET. Among the biological parameters are cell type (e.g., fibroblastic, hemopoietic, epithelial cells), state of proliferation and/or differentiation, repair capability, tissue of origin (for studies *in vitro*), and tissue organization (for studies *in vivo*). In addition, as noted in Subsection 3.4.4 and discussed below, to develop estimates of how radiation induces the different steps in tumorigenesis, answers to questions are needed that are inherently biological and not specifically connected with radiation effects.

3.5.3.1 Oncogene Activation

Whatever the inducing process, an oncogene may be caused to act dominantly in a number of different ways (see Task 2 and Subsections 3.4.1 & 2). Mutations resulting from point changes in the structural oncogene itself, or in its regulatory elements, can give rise to a qualitatively altered product, or to alterations in the amount of an otherwise normal oncogene product. Altered regulation could result from a chromosomal rearrangement as illustrated by Burkitt's lymphoma. Activations that result in altered proliferation and/or differentiation can be the basis for the clonal expansion of a progenitor cell.

In instances where an oncogene is activated indirectly--that is, as a result of changes induced in other genetic elements such as *trans*-acting regulatory elements--the frequencies of induction may not bear a predictable relationship to the size of the oncogene and/or its *cis*-acting control elements as radiation targets. In such instances, the probability of induction of the change would be expected to be different from that involving independent

radiation action in the oncogene because the change would be conditional upon other genetic or epigenetic events. The latter situation is illustrated by the chromosomal rearrangements in Burkitt's lymphoma, which are believed to lead to the enhanced expression of the *c-myc* oncogene.

A further feature of "conditional" activation is that the cell in which the precipitating event is expressed may give rise to an activated cell only after some amount of time and/or cell division. The reason is that metabolic and replicational processes may have to ensue before the oncogenic activation can be effected. For example, activation of an oncogene could result from a point mutation that was a consequence of a greater probability of miscoding during the replicative synthesis of that gene. In such an instance, time, metabolism, and replication may be required before the mutated oncogene is produced.

In addition to activations connected with oncogenesis, a second requirement of such mutations is that cell viability should be maintained even if a growth advantage is not induced. When the nature of the mutation involves localized changes--such as, point mutations, intragenic deletions or insertions--on monosomic chromosomes, it is likely that viability will be maintained because the flanking regions will remain functional. The conditions for the maintenance of viability are less stringent for similar mutational events on autosomes although it has been reported that the loss of essential genetic information on one chromosome of a homologous pair can result in a growth disadvantage (see DeMarini *et al.*, 1989).

3.5.3.2 Oncogene Inactivation

The inactivation of oncogenes is associated primarily with the recessive, anti-oncogene/suppressor-gene mechanism of tumorigenesis. In familial cancer, at least one somatic mutation in the homologous allele is inherited by the fetus. Because only an inactivating change is required in the homologous gene of the offspring, a number of different genetic alterations have the potential of being effective. These include: intragenic point mutations, deletions, and insertions; changes in DNA sequences that reduce the effectiveness of control elements; deletions of part of the chromosome in which the suppressor gene is located; chromosomal rearrangements, for example due to recombination or translocation, that result in an inactive gene; loss of the entire gene because of nondisjunction involving the chromosome in which the gene is located; and gene conversion, the inactivation of the normal allele by an intragenic interchange with the inherited, mutated allele (Liskay *et al.*, 1984; Wang *et al.*, 1988). In each of the foregoing, the attendant loss of heterozygosity would have to result in a growth advantage for the cell in which both anti-oncogenes had been inactivated or rendered nonfunctional. Although the probability of gene conversion between homologous chromosomes is likely to be low, in principle it also can be a mechanism whereby an intragenic mutation could become homozygous in a somatic cell. Consequently, gene conversion could lead to the loss of heterozygosity in familial and nonfamilial cancer providing that the conversion of the nonmutated allele confers a growth advantage on the cell.

Thus, inactivation of an anti-oncogene could result from a number of different kinds of mutagenic events. From this fact alone, it would appear to be more likely that neoplastic transformation caused by radiation would result from autosomal allelic loss or inactivation as opposed to the activation of an oncogene. The larger targets represented by chromosomal, as opposed to genetic

elements, also would suggest that with radiation anti-oncogene inactivation would be favored over oncogene activation.

In the foregoing connection, certain caveats should be noted in reference to the quality and duration of the radiation exposures. Radiation of high-LET is even more likely than radiation of low-LET to produce chromosomal changes rather than point mutations at the same levels of survival (Thacker, 1985), and ordinarily repair processes are less effective in modifying high-LET- vs. low-LET-induced endpoints. In addition, reducing the dose rate of a low-LET radiation may result in a shift in the spectrum of mutations toward more discrete genetic changes as exposures are extended in time. If such a shift occurs, the genetic changes would be expected to be more like those produced by chemical carcinogens.

3.5.3.3 Independent vs. Conditional Oncogenic Events

In reference to solid tumors, as opposed to those of the blood-forming organs, in general a larger number of genetic alterations appear to be involved. Some five changes or more are associated with colorectal carcinoma, and perhaps more than 10 in some lung neoplasms. In the instance of lung cancer, it has been documented that smoking--especially when associated with radon in the atmosphere (BEIR IV Report, 1988)--plays a significant role in its incidence and that the degree of risk increases significantly with the length of the smoking experience. Hence, in such instances the large number of oncogenic changes could bear a relationship to the duration and intensity of exposure to the inducing, or promoting, agent. Still, the large numbers of changes that have been identified particularly in solid tumors raises the question: Which changes are causes and which are effects (or are coincidental), and, hence, which are independent and which are conditional mutagenic events (i.e., dependent upon some other event)?

If the expression of a particular oncogenic change is conditional upon the occurrence of some other oncogenic event, then the probability of the induction of the change by radiation could be significantly different from that corresponding simply to its target size. The net probability would be significantly smaller than indicated by the target size if, for expression, both changes have to be effected independently. The net probability could be significantly larger than the latter value if, in the limit, the conditional change does not require radiation induction but is a consequential effect of the independent event. For example, the conditional change might result from an error in the replicational/division processes of the irradiated cell. It may take time for this error to occur but, if it finally confers a growth advantage, the conditional change will be expressed because the cell will be selected for (see Fig. 2.7).

Hence, a low radiation-induced incidence of a given neoplasm could reflect the requirement that several, independently registered radiation events are required. If five or more independent changes are involved, as is thought to be the case in colorectal carcinoma, the probability (or target size) of each might even be relatively large but the net probability would still be small. If all five changes are required for the carcinoma to be expressed, the probability of expression (target size) of those changes that are independent would depend on the expression of the changes that are conditional or dependent.

Lastly, target sizes as discussed above are equal to the physical sizes of molecules, or molecular structures, when repair/misrepair processes are without effect. Generally speaking, following a low-LET exposure at a high dose rate, repair can be expected to reduce the effective, vs. the physical, size of the target, whereas little or no repair would be expected following a high-LET exposure. At low dose rates, repair may further reduce effective target sizes after a low-LET exposure with no change, or even an increased effective target size (misrepair), after a high-LET exposure (see Subsections 3.2.1 & 2).

3.5.3.4 Necessary vs. Sufficient Requirements

Although in studies with inbred rodents, it has been possible to show that the chemical mutation of, apparently, a single oncogene, the *ras* gene, was able to induce neoplasia (Barbacid, 1986), and although similar inferences have been made in reference to radiation-induced neoplasia (Guerrero et al., 1984a,b), the plethora of oncogenic changes that have been associated with human tumors prompts the question: Which oncogenic events are necessary and which are sufficient for the induction of neoplastic growth? A related question is: If not all of the oncogenic changes are necessary, are there changes that are consequential to a particular necessary change but are neither necessary nor sufficient by themselves? As in the preceding subsection, these questions are also basic to the mechanism of neoplasia and, consequently, they could significantly influence estimates of frequencies of radiation-induced cancer derived from first principles.

3.5.4 Elements of a Program of Research

Reduction of the uncertainties of radiation-induced neoplasms requires a knowledge of the radiation-induced probabilities that cause the oncogenic changes. It is evident from Section 3.4 that a number of changes are associated with specific human neoplasms and that these changes are not entirely unique in a given case. Which of these changes are necessary is not clear, however.

If the growth states for a given neoplasm are fully defined, if the necessary and sufficient requirements are understood, and if uncertainties about independent vs. conditional (including derivative) genetic changes are resolved, it should then become possible to estimate dose dependencies to effect transitions from one growth state to the next. Thus, in principle, the dose dependence of the overall process could be computed from the probabilities of effecting each transition. The latency period, which depends on the particular neoplasm in question, has to be accounted for. These periods may be as short as a few years, in the instances of hemopoietic neoplasms, to decades for some solid tumors. The number of oncogenic lesions in each growth state, and the selective outgrowth of the transformed phenotype, may determine the length of these periods, or the latency periods may depend upon the rate of accumulation of the lesions followed by the selective outgrowth of neoplastic phenotypes (see Fig. 2.7).

Without a prioritization intended, the elements of a research program are listed below. The neoplasms that would be of primary interest are those that have been identified as radiogenic, such as: acute and chronic myeloid leukemia; acute lymphocytic leukemia; and carcinomas of the breast, colon/rectum, lung, and thyroid (see BEIR V Report, 1990).

1. The gaps identified in Subsection 3.4.4 need to be eliminated. Much of this information will be forthcoming from molecular biologists who may not be especially concerned with radiation-induced cancer.
2. Growth-state characterizations are needed for radiogenic human neoplasms. A logical consequence of a basic research program in radiation-induced oncogenesis would be that radiobiologists would also participate in developing this information.
3. The mechanisms of how transitions between growth states are effected has to be determined. Scientists studying human oncogenesis in general, as well as those participating in a radiation research program, should contribute to this objective.
4. The activation and inactivation of cancer genes by radiation should be determined as well as how these processes depend upon dose, dose rate, dose protraction, and LET. Because these changes frequently may involve chromosomal alterations, the mechanism of the production of such aberrations should be determined. High-LET studies could help to distinguish primary from derivative oncogenic changes. Fission-spectrum neutrons could serve as a substitute for a high-LET beam, and, consequently, the data obtained would be useful in reference to the radiation exposure of industrial and military personnel.
5. Suitable cell and animal systems should be developed for the measurement of activation/inactivation probabilities *in vitro* and tumor induction *in vivo*. One of the objectives in the use of these systems would be to test the principles that are needed to predict the induction of particular growth states based upon measurements of the probabilities of the oncogenic changes that characterize a growth state in a particular neoplasm.
6. Initially using cell systems, possible interactive effects should be determined between radiation and other genotoxic physical and chemical agents that are found in the environment. This need stems from the possibility that other environmental agents may significantly promote initiation events due to radiation (e.g., Hill et al., 1989).
7. In addition to the basic radiation-induction measurements noted in point 4 above, data from animal systems in reference to specific neoplasms may be extrapolatable to people. This possibility is illustrated by the work of Nomura (1982)--who studied the transmission of cancer susceptibility to the offspring of mice irradiated before conception--and the possible relevance of his results to the epidemiological data of Gardner et al., (1990) on the relation of the induction of leukemia in children to parental exposure. Hence, the principles with which to validate the extrapolation of animal data to people should be developed via research.

3.6 EXPERIMENTAL SYSTEMS

The information that is required to make feasible an understanding of mechanisms of radiation-induced cancer should make extensive use of cellular systems and, as appropriate, cell-animal and animal systems. These systems would serve three main purposes.

First, they would facilitate the measurement of the dependence on radiobiological parameters of the kinds of genetic changes that are associated with human neoplasms (see Section 3.4).

Second, they would enable comparisons to be made between the measured dose dependencies of radiation tumorigenesis in animals and the dependencies predicted on the basis of cellular/molecular measurements.

And third, they would serve to develop a rational method of extrapolating biologically from rodents, for which the most data are available, to man at the levels of *in vitro* and *in vivo* endpoints.

The overall objective of the research with experimental systems would not be to develop quantitative estimates of oncogenic risks for animals as such, but rather to use such data to elucidate the basic principles and mechanisms of the oncogenic process. Experimental systems would be used to determine the relationship between the oncogenic mechanisms in animal and human cells, and tissues.

3.6.1 Cell Systems in Culture

As indicated by the considerable progress achieved thus far in research on somatic mutations, tumor viruses, and oncogenes, many of the issues that were raised in Sections 3.4 & 3.5 can be explored using animal or human cells in culture. Several endpoints are available that constitute so-called *in vitro* correlates of unregulated growth *in vivo* although additional indicators for this purpose are needed. In addition, to test for tumorigenic potential, the cells in question may be inoculated into suitable hosts, including immunologically deficient mice, for assays with human cells.

The usefulness of cell systems in culture would be increased by technical improvements along the following lines.

1. Additional endpoints of mutation and neoplastic transformation are required that are amenable to accurate, quantitative measurements at molecular and cellular levels. The low frequencies of phenotypic change, particularly when low doses are used, is a barrier to accuracy. The correlation of *in vitro* endpoints with the transformations consequent to oncogenic change *in vivo* should be established. Methods are required to enable working with primary as well as with immortalized cells, and with cells that are representative of the tissues in which radiation is known to be tumorigenic.
2. Various animal sera are used to fortify the media for the cultivation of cells. Because the compositions of sera are not fully defined, fluctuations are generally encountered in the properties of media. Chemically defined culture media are required, which simulate the conditions of growth *in vivo*, and which permit reproducibility of results.
3. Other physical as well as chemical environmental agents should be examined to determine if they affect, increase or decrease, the yields of radiation-induced molecular and cellular endpoints related to neoplastic transformation. An example of this possibility comes from the enhancement

by a chemical promoter of neoplastic transformation induced at a low dose rate (Hill et al., 1989).

4. Most of the data on radiation-induced transformation are from studies performed with rodent cells. To enable a biological extrapolation from the data with rodents and other species to man, representative cell cultures, for example, from dog, monkey, and human tissues, would be required.

3.6.2 Animal vs. Human Neoplasia

The use of animal model systems for research on radiation oncogenesis should be understood in the context of how and where they can be applied. The thrust of the research should be to bring to bear knowledge made possible by the new methods of cellular and molecular biology. If the basic processes of oncogenesis can be shown to be similar between species, then the flexibility of animal models can be used to advantage in addressing questions of relevance to human risks.

For example, as described in Section 3.4, it is becoming clear that multiple genetic events are involved in the development of several of the most common human neoplasms. It is not yet clear whether this may be true of histologically similar animal neoplasms, but there are indications that such may be the case (Hanahan, 1989; Silver et al., 1990; see also Subsection 3.6.3). There are only limited data, for either radiation-induced human or animal neoplasms, on the nature and extent of the genomic changes that are associated with oncogenesis. For animal tumors, efforts to evaluate the sequential genetic changes and their associated growth states are underway but they lag behind the progress that has been made with human neoplasms.

The foregoing points to an essential gap in our knowledge. It is not known if, and to what degree, radiation-induced neoplasms, in corresponding animal and human tissues, show the same oncogenic characteristics. To begin with, at the present it is not possible to identify individual radiation-induced human neoplasms. In addition, the oncogenic characterizations are incomplete, particularly for animal tumors. However, it has been demonstrated that oncogenes from many species are at least similar in both structure and function. Human oncogenes have been shown to cause the same tumors in mice as the tumors from which they were derived (Hanahan, 1989). If it can be shown that the oncogenic changes in spontaneous animal neoplasms are similar to those in spontaneous human neoplasms, the usefulness of animals in comparative studies would be supported. The arguments for basing a radiation research program of human cancer on the genetic features of spontaneous tumors (see Subsection 3.5.1) would also apply to animal tumors.

3.6.3 Animal Models

To facilitate studies of the mechanism of radiation-induced neoplasia in animals, advantage should be taken of the attributes of several systems. These include organs and tissues that have been shown to be sensitive to radiation oncogenesis in several species as well as in people (see 3.5.4). The systems to be discussed are not the only possible models; however, as noted below, they appear to hold the greatest potential for yielding quantitative and relevant data.

3.6.3.1 Leukemia/Lymphoma in Mice

One of the most promising models for the study of oncogenes and growth factors in radiation oncogenesis would be neoplasms of the hematopoietic tissues of mice. In murine radiation leukemogenesis, changes in the regulation of growth factors, e.g., interleukin-1 (*IL-1*), have been identified that could be important in the control of marrow-cell proliferation (Griffen *et al.*, 1987; Silver *et al.*, 1990). As noted in Subsection 3.3.4.3, these changes are associated with rearrangements in (mouse) chromosome 2 (Silver *et al.*, 1990; Haran-Ghera, 1989) although other changes, such as promotion by corticosteroids, may also be involved (Haran-Ghera, 1989). In analogy with the genetic characteristics of human leukemia, multiple changes also may be involved in rodent leukemia.

It is noteworthy that some apparent differences have been identified in the chromosomal changes between spontaneous and radiation-induced leukemias in mice (Haran-Ghera, 1989). Also, acute myeloid leukemia cells, in several strains of mice, have been found to have normal-appearing diploid karyotypes although it is noted that the microscopic analysis of mouse chromosomes is not as advanced as that of human chromosomes. Further, B-cell lymphomas in the same group of irradiated animals that developed myeloid leukemias did not show the rearrangements of chromosome 2. It remains to be determined whether or not the difficulty in distinguishing changes in mouse chromosomes by karyotype banding, compared to human chromosomes, is the reason for the instances of apparently normal-appearing karyotypes in lymphomas. With human leukemia cells, significant but subtle karyotypic changes were detected in all instances when techniques became sufficiently improved (Yunis *et al.*, 1981; see Subsection 3.4.2). In reference to the observation of Haran-Ghera (1989) above, it also remains to be determined if differences in chromosomal rearrangements in cells from radiation-induced vs. spontaneous leukemias will persist as the dose is reduced and/or the length of the exposure increased.

From the information that is available, a model for leukemogenesis in mice would be as shown in Fig. 3.9. Although differences exist between the information in Fig. 3.9 and what is summarized in Figs. 3.4 & 3.5 for human lymphoma and leukemia, there are similarities. Hence, an understanding in murine leukemia of the roles of major chromosomal rearrangements, the regulation of growth factors, and how they are affected by radiation should be applicable to the human situation.

3.6.3.2 Pulmonary Neoplasia

The lung is an important target organ for oncogenesis in humans due to radiation and other inducing agents. The model rodent systems developed for the study of radiation oncogenesis in the lower respiratory tract (trachea, bronchi, and lungs) has yielded insights that suggest that the model is appropriate for further study.

Mice, rats, and dogs have been used extensively for studies of pulmonary radiation oncogenesis *in vivo*, and more recently, the latter two species have been employed in *in vivo/in vitro* cell studies. From the standpoint of *in vivo* research, genetic susceptibility in the mouse pulmonary-adenoma system has been shown to be under control of at least three genes, including the proto-oncogene, *K-ras-2* (Malkinson, 1989).

Fig. 3.9 Growth states, and the associated cellular/chromosomal changes, that have been identified in mouse, acute myeloid leukemia.

Recent studies have utilized both rats and dogs to evaluate the expression of oncogenes and their products. In lung neoplasms induced by α -particles emitted from inhaled ^{239}Pu , canine lung neoplasms, squamous-cell carcinomas and papillary adenocarcinomas, were found to have amplified expressions of the receptor of epidermal growth factor (Gillett et al., 1989) which has also been reported for human lung carcinoma (Cerny et al., 1986; Berger et al., 1987; Dazzi et al., 1989). Normal canine lung tissue did not express the receptor but it was expressed by a low percentage of hyperplastic, putatively preneoplastic, foci (Gillett et al., 1989). In addition, the expression of the oncogene *erbB*, which codes for part of the epidermal growth factor, was increased. However, the receptor was expressed in both α -particle-induced and spontaneous canine lung neoplasms in contrast to the situation in leukemia in mice where a oncogene change specifically induced by the radiation has been evident. To be clarified, is whether or not radiation-induced lung neoplasms in rodents have an enhanced expression of the receptor as in dogs (Stegelmeyer et al., 1989). Lung tumors in dogs may have some advantages for study because of their expression of the receptor for epidermal growth factor and their histological similarity to human lung tumors.

An *in vitro/in vivo* model system has been developed for the rat for studying neoplasms of the respiratory system that also holds promise for effective use in radiation studies. After radiation or chemical treatment, there appear to be multiple steps in the overall oncogenic process with the appearance of preneoplastic followed by fully neoplastic cells (Terzhagi et al., 1989). Resistance to transforming growth factor- β has been identified as an early step in the mechanism by which transformed respiratory cells escape terminal differentiation. Recent work with tracheal cells has compared the ability of X-rays and neutrons to induce preneoplastic changes in tracheal cells exposed as suspensions or as intact tracheas (Terzaghi-Howe, 1989). Neutron exposures effectively induced preneoplastic changes in both situations, but X-rays did so only for cells in suspension. With X-rays, the lack of a response when intact tracheas were exposed suggests that cell-to-cell communication and/or tissue interactions affected the transformation process.

The *in vitro/in vivo* system referred to above uses rat tracheal epithelial cells. However, most radiation-induced neoplasms occur either in the bronchi or deeper in the lung. In order to develop a model system for the more appropriate target cells in lung neoplasia, a system based on a larger animal may be required. A transformation system using tracheal epithelium from the dog has been developed recently (Hubbs et al., 1988). Another possibility would be to examine the usefulness of a xenograft, cell-animal system in which cells from human epithelium of the lower respiratory tract would be treated and then assayed by implantation into cell-depleted trachea of immunologically suppressed rodents.

3.6.3.3 Mammary and Thyroid Neoplasia

In vitro/in vivo studies using cells of the mammary and thyroid glands of the mouse and the rat have shown that radiation-induced neoplasms arise from a subpopulation of clonogenic epithelial cells (see Subsections 1.4.4 and 1.4.5). These studies suggest that initiation may be a relatively frequent change compared to the incidence of neoplasia (Clifton, 1986; Clifton et al., 1986). This type of *in vitro/in vivo* model system lends itself to examining the relationship between the neoplastic changes induced in cells by an oncogenic agent and the tumors that would be produced. In this connection, it is noted

that both mammary and thyroid tissues are sensitive to radiation-induced neoplasia (see Subsection 3.5.4).

Information along the foregoing lines should be possible for a wider variety of animal cell types and species. A number of questions could be asked. Do cells from radiosensitive tissues show higher transformation rates than do other cells? If not, are the differences in tumor rates due to hormonal, immunologic, or viral influences? Are there essential similarities or differences between cells from the same tissues but different species? Do the oncogenic changes in progenitor cells vary with the age of the host?

3.6.3.4 Transgenic Mice

Transgenic mice have potential for research on mechanisms of neoplasia (Pattengale et al., 1989; Hanahan, 1989; see also Section 2.5). A considerable number of oncogenes have been incorporated into transgenic mice which has facilitated the study of the specific oncogenic activity associated with each in an *in vivo* environment.

One pattern that has emerged in such animals has been the development of hyperplastic lesions prior to the appearance of histologically recognizable neoplasms (Hanahan, 1989). Oncogenes identified by, or associated with, increased proliferation *in vitro* also elicited hyperplasias in transgenic mice suggesting that deregulation of growth controls is a major contribution to oncogenesis by these genes. However, in transgenic mice, with many oncogenes an increased proliferative state alone is not sufficient to induce a full-blown neoplasm. Multiple steps have been found to be required, some of which have been identified (Hanahan 1989; Pattengale et al., 1989), as have been described in human neoplasms (see Section 3.4).

Using DNA recombinational techniques, oncogenes may be fused to tissue-specific promoters and/or enhancer sequences that direct the oncogenes to specific tissues. For example, the human *c-neu* oncogene fused to the promoter of the mouse mammary tumor virus becomes potently oncogenic in the mammary glands of transgenic mice (Pattengale et al., 1989). The human homolog *c-neu*, also called *c-erbB2*, is amplified and expressed in human breast cancer (van de Vijver et al., 1987). These findings suggest an important role for the human *c-neu* gene in breast cancer.

Other studies have shown that a human immunoglobulin gene reduces the incidence of B-cell lymphomas suggesting that the action of tumor-suppressor genes could be evaluated with transgenic mice (Nussenzweig et al., 1988). Thus, transgenic mice may facilitate the study *in vivo* of transitions from the normal to the neoplastic state, research that may be difficult to accomplish by other means.

In practical terms, thus far the transgenic technology has been limited largely to mice. Methods of gene transfer using larger animals, which are under development in reference to other areas of work, could facilitate the application of this technology to studies of oncogenesis.

3.6.4 Extrapolation to People

With the exception of human cells in culture and the use of xenografts to assess when they have become neoplastically transformed, in general, animal-based

experimental systems suffer from the limitation that the findings may correspond only more or less well to the human situation. Mice are not men. Still, many similarities connect mammalian species suggesting that the problem of extrapolation from experimental systems to people could be examined by two parallel approaches.

Cellular Studies: Extrapolation from cellular data to the various kinds of tumors that occur in people, should, in principle, require the use of cell systems derived from *ectodermal*, *endodermal*, and *mesodermal* tissues. Compromises could be made to limit the cell types to those that correspond to the most frequent human neoplasms, i.e., epithelial cells *vis-a-vis* carcinomas. To determine if and how results obtained with rodent cells in culture apply to human cells in culture, cell systems would be required from species that would permit a biological extrapolation to human material. In determining which species to use, consideration should be given to metabolic rates, developmental characteristics, life span (as well as to the existence of corollary information on the natural incidence of cancer), and the amount of data that are available on experimental oncogenesis. Considerations such as the foregoing suggest the dog and the monkey in addition to rodents, i.e., mice, rats and hamsters.

Animal Studies: The second approach would be to develop parallel information on tumorigenesis in different species. To maintain coherence in the extrapolation procedure, the same species should be used as in the cellular work. The sites of interest should be limited to those whose frequency makes them of relevance in human neoplasia. However, the ability to manipulate experimental systems invites the examination of oncogenic mechanisms in general even when the tumor type in question may not be of principal concern to human disease.

Thus, conceptually the kinds of studies outlined in Subsection 3.5.3 should be applied to those cells and tissues in mammalian species that would support rational extrapolations from rodents to people. These investigations should include: the measurement of the frequencies of the radiation-induced oncogenic changes representative of dominant and recessive oncogene action, as well as the associated mutation and chromosomal effects; the testing of predicted vs. observed frequencies of radiation-induced neoplasms; and the examination of the fundamental mechanisms of oncogenesis. The method of biological extrapolation could lead to judgments of how experimental cell and animal data should be developed to improve their usefulness in predicting human disease.

3.7 SUMMARY

This Task has four essential parts. The first is a critical review of the information that is available, primarily from cell and animal studies, that is connected with mechanisms of radiation-induced oncogenesis. The second is a review of what has been discovered of the roles of genes, particularly oncogenes, in radiation-induced endpoints. The third is an analysis of what is known about the role of oncogenes in human neoplasia. And the fourth is a prospectus of the scope of a radiation research program to reduce the uncertainties of the risks of cancer from low doses of radiation. In addition, gaps in our knowledge are identified in reference to the topics that are important to the understanding of radiation-induced cancer.

3.7.1 The Status of Information on Cellular Neoplastic Transformation and Tumorigenesis in Experimental Systems

Although the kinds of cells that have been available for experimental work are limited, significant insights have emerged connecting the radiobiology of neoplastic transformation *in vitro* and tumorigenesis *in vivo* as follows:

- 1) The dose dependence of tumor induction reflects a competition between the transformation events and the lethal events in the target cells of a given tissue (Fig. 3.1 and 3.2). To be the progenitor of a population of cells that constitute a neoplasm, an irradiated cell must survive. In a number of instances, the net effect of these two processes is a bell-shaped curve of the dependence of tumor induction on dose (Fig. 3.2).
- 2) As for other endpoints, the efficiency of the neoplastic transformation of cells, and the induction of tumors, increases with LET. In general, a high-LET radiation produces larger frequencies per unit dose than does a low-LET radiation. However, because cells are generally killed more efficiently with a high- vs. a low-LET radiation, cell killing can have a more pronounced effect with the former. Thus, the bell-shaped curve of tumor induction with a high-LET radiation is generally shifted toward lower doses compared to that with a low-LET radiation (Fig. 3.2).
- 3) In addition to the influence of repair relative to cell killing, repair/misrepair can significantly alter the radiation inductive process. In the low dose region, where cell killing is minimal, reducing the rate of exposure to a low-LET radiation generally results in a reduction in the initial slope of the cell transformation or the tumor induction curve. With low dose rates of a high-LET radiation, such as fission-spectrum neutrons, the situation is less general. In some instances, protracting the exposure has increased yields of transformed cells or tumors, and in others it has not or it has reduced them. A generalization in respect to the influence of dose protraction of high-LET radiations is not possible at the present time.
- 4) Although the initial yield of neoplastically transformed cells is significantly reduced at reduced rates of a low-LET exposure, the net yield of transformants can be appreciably increased by the action of a compound known as a promoter, e.g., a phorbol ester, applied after the irradiation. Hence, the possibility exists that other agents in the environment may interact with radiation effects to alter the yield of transformed cells and, therefore, tumors due to a given radiation experience.

The analysis of *in vitro/in vivo* data made evident a number of gaps in our knowledge and the principal deficiencies were discussed.

3.7.2 The Involvement of Genes and Oncogenes in Radiation Endpoints

The power of the genetic methods that has emerged from the application of DNA recombinant techniques in other areas of research led quite naturally to the application of these methods in radiobiology. Some progress has been made, but the amount of information available thus far is limited. The current situation is evident from the following.

- 1) The survival of cultured cells can be influenced by the *ras* oncogene, but the basis for this effect has not been worked out. Similarly, it appears that the application of a growth factor, like epidermal growth factor, can alter the survival response of certain cells.
- 2) Radiation is an effective mutagen. However, in contrast to chemical mutagens, the heritable changes that are produced by radiation generally are due more to chromosomal breakage-rearrangement events, *extragenic mutations*, than to point effects, *intragenic mutations*.
- 3) Although specific genes appear to be induced by radiation in transformed cultured cells, they do not appear to be among the known oncogenes.
- 4) Progress has been made on the roles of some oncogenes in tumors induced in experimental animals particularly in respect to acute myeloid leukemia in the mouse. The need for the activation, in some instances, of at least two oncogenes has also been noted.

In reference to the endpoints discussed above, a number of gaps were evident and these were discussed as subjects for additional research.

3.7.3 Human Neoplasms and Their Growth State Characterizations

The fruitful application of the methods of contemporary molecular biology are no more evident than in the study of oncogenes in human cancer. Significant advances have been made in characterizing, in certain types of tumors, the mechanism of action of those oncogenes that appear to be activated as well as in locating them in the chromosomes of somatic cells (see Tables 2.1, 2.3, and Fig. 2.3). The following points were brought out:

- 1) Chromosomal rearrangements and/or deletions, as well as the activation of particular oncogenes, are involved in specific spontaneous neoplasms. The oncogene/chromosomal data support the hypothesis that human tumors are clonal in origin.
- 2) A correlation appears to exist between the number of oncogenic/chromosomal alterations in a given type of neoplasm and the latency period for its appearance; tumors that have few changes appear earlier than those that have many changes.
- 3) In some instances, oncogenic/chromosomal characterizations have been sufficiently worked out to facilitate an association between specific histological states with specific characterizations, e.g., Burkitt's lymphoma, chronic myeloid leukemia, and colorectal carcinoma.
- 4) Some human tumors are believed to require a number of essential changes. As a consequence, it is not clear what is cause and what is effect and, therefore, which changes are independent and which dependent.

Although the progress in characterizing spontaneous human neoplasms is significant and its rate accelerating, what has been accomplished thus far in most instances is to describe rather than to explain. Thus, the oncogenic/chromosomal descriptions are a kind of taxonomy. In addition to the need for similar characterizations of additional radiogenic human tumors, a number of fundamental questions are evident. Principal among these is if and how

successive growth states are produced by radiation. This gap and others are identified.

3.7.4 Prospectus of a Radiation Research Program

Numerous factors, in addition to those that are radiobiological, are known, or suspected, to play a role in influencing radiation oncogenesis. In sum, these can be expected to underlie uncertainties in the epidemiology of cancer including radiation-induced cancer. The recently issued BEIR V Report (1990), in which estimates of some radiation risks were significantly increased, illustrates the need to modify earlier estimates because of the lack of a coherent set of ideas of how cancer is induced by various etiological agents including radiation. In view of the apparent complexity of the problem, one must inquire: Is it feasible to reduce the uncertainties of radiation-induced neoplasms based upon an expanded knowledge of the underlying mechanism(s) of the induction of unregulated cellular proliferation *in vivo*?

The answer to the foregoing question, which is developed in Sections 3.5 and 3.6, is a qualified "yes." This optimism is based largely on: 1) the dramatic developments that are occurring in the understanding of the oncogenic basis of human cancer; 2) the assumption that growth-state characterizations will be completed for all of the radiogenic human cancers of interest; and 3) the assumption that the molecular biology of the radiation-induced transitions between growth states will become understood.

In addition, to accomplish a significantly improved understanding of radiation oncogenesis, so that the uncertainties in radiation risks may be reduced, three principal objectives are identified:

- a) A mechanistic framework is needed to relate, in a rational way, radiation-induced neoplasia to spontaneous cancers;
- b) Growth-state characterizations, and the radiobiology of transitions between them, will have to be worked out for animal tumors just as is being done for human neoplasms; and
- c) Strategies must be validated that will enable extrapolation of experimental data on tumorigenesis to people.

Although differences are anticipated between the oncogenic changes in cells that culminate in human as opposed to animal cancer, it is quite likely that essential similarities exist. Thus, for example, the oncogenic basis for the uncontrolled growth of animal and human myeloid cells, which are manifest as leukemia, is expected to result from changes that are similar in essence. Hence, understanding the mechanism of mouse leukemia should facilitate the understanding of human leukemia.

3.8 REFERENCES

- Balcer-Kubiczek, E. K., G. H. Harrison, G. H. Zeman, P. J. Mattson, and A. Kunska, "Lack of a Dose Rate Effect on Fission-Induced Transformation of C3H 10T1/2 Cells." International Journal of Radiation Biology 54, 81-89, 1988.
- Balcer-Kubiczek, E.K., G. H. Harrison, and B. W. Thompson, "Repair Time for Oncogenic Transformation in C3H/10T1/2 Cells Subjected to Protracted X-Irradiation." International Journal of Radiation Biology 51, 219-226, 1987.
- Balmain, A., and I. B. Pragnell, "Mouse Skin Carcinomas Induced *in Vivo* by Chemical Carcinogens have a Transforming Harvey-ras Oncogene." Nature 303, 72-74, 1983.
- Barbacid, M., "Oncogenes and Human Cancer: Cause or Consequence." Carcinogenesis 7, 1037-1042, 1986.
- BEIR IV Report, "Health Risks of Radon and Other Internally Deposited Alpha-Emitters," National Academy Press, Washington, D. C., 1988.
- BEIR V Report, "Health Effects of Exposure to Low Levels of Ionizing Radiation." National Research Council, Washington, D.C., 1990.
- Berger, M. S., W. J. Gullick, C. Greenfield, S. Evans, B. Addis, and M. Waterfield, "Epidermal Growth Factor Receptors in Lung Tumors." Journal of Pathology 152, 297-307, 1987.
- Birrer, M., and J. Minna, "Genetic Changes in the Pathogenesis of Lung Cancer." Annual Reviews of Medicine 40, 305-317, 1989.
- Boice, J. D., G. Engholm, R. A. Kleinerman, M. Blettner, et al., "Radiation Dose and Second Cancer Risk in Patients Treated for Cancer of the Cervix." Radiation Research 116, 3-55, 1988.
- Borek, C., A. Ong, and H. Mason, "Distinctive Transforming Genes in X-Ray-Transformed Mammalian Cells." Proceedings of the National Academy of Sciences USA 84, 794-798, 1987.
- Borek, C. and L. Sachs, "In Vitro Cell Transformation by X-Irradiation." Nature 210, 276-278, 1966.
- Borek, C., "Neoplastic Transformation Following Split doses of X-Rays." British Journal of Radiology 50, 845-846, 1979.
- Breckon, G., A. R. J. Silver, and R. Cox, "Consistent chromosome changes in Radiation-Induced Murine Leukemias." In: Kew Chromosome Conference III, eds., P. E. Brandham and M. D. Bennett, HMSO, London, pp 179-184, 1988.
- Burck, K. B., E. T. Liu, and J. W. Larrick, eds., Oncogenes: An Introduction to the Concept of Cancer Genes, Springer-Verlag, New York, 1988.

- Burns, F. J., and M. Vanderlaan, "Split-Dose Recovery for Radiation-Induced tumors in Rat Skin." International Journal of Radiation Biology 32, 135-144, 1977.
- Callahan, R., G. Campbell, "Mutations in Human Breast Cancer: An Overview." Journal National Cancer Institute 81, 1780-1786, 1989.
- Cerny, T., D. M. Barnes, P. Haselton, P. V. Barber, K. Healy, W. Gullick, and N. Thatcher, "Expression of Epidermal Growth Factor Receptor in Human Lung Tumors." British Journal of Cancer 54, 265-269, 1986.
- Cornforth, M. N., and J. S. Bedford, "X-Ray-Induced Breakage and Rejoining of Human Interphase Chromosomes." Science 222, 1141-1143, 1983.
- Clifton, K. H., "Thyroid Cancer: Reevaluation of an Experimental Model for Radiogenic Endocrine Carcinogenesis." In: Radiation Carcinogenesis, eds., A.C. Upton, R.E. Albert, F.J. Burns, and R.E. Shore, Elsevier, New York, pp 181-198, 1986.
- Clifton, K.H., M. A. Tanner, and M. N. Gould, "Assessment of Radiogenic Cancer Initiation Frequency per Clonogenic Rat Mammary Cell *in Vivo*." Cancer Research 46, 2390-2395, 1986.
- Curtis, S. B., "A Possible Role of the Inverse Dose-Rate Effect in the Radon Exposure Problem." In: Low Dose Radiation, eds., K. F. Baverstock and J. W. Stather, Taylor & Francis, London, pp 547-553, 1989.
- Dazzi, H., P. S. Haselton, N. Thatcher, D. M. Barnes, S. Wilkes, R. Swindell, and R. A. M. Lawson, "Expression of Epidermal Growth Factor Receptor in Non-Small Cell Lung Cancer: Use of Archival Tissue and Correlation of EGFR with Histology, Tumor Size, Node Status, and Survival." British Journal of Cancer 59, 746-749, 1989.
- DeMarini, D. M., H. E. Brockman, F. J. de Serres, H. H. Evans, L. F. Stankowski, Jr., and A. W. Hsie, "Specific-Locus Mutations Induced in Eukaryotes (Especially Mammalian Cells) by Radiation and Chemicals: a Perspective." Mutation Research 220, 11-29, 1989.
- Diamond, L. E., I. Guerrero, and A. Pellicer, "Concomitant K- and N-ras Gene Point Mutations in Clonal Murine Lymphoma." Molecular and Cellular Biology 8, 2233-2236, 1988.
- Elkind, M. M., A. Han, C. K. Hill, and F. Buonaguro, "Repair Mechanisms in Radiation-Induced Cell Transformation." In: Radiation Research, Proceedings of the 7th International Congress of Radiation Research, eds, J. J. Broerse and G. W. Barendsen, Martinus Nijhoff, Amsterdam, 33-42, 1983.
- Elkind, M. M., H. Utsumi, and E. Ben-Hur, "Are Single or Multiple Mechanisms Involved in Radiation-Induced Mammalian Cell Killing?" British Journal of Cancer 55, Supplement VIII, 24-31, 1987.
- Elkind, M. M., "Cell Killing in Radiation Tumorigenesis." In: Radiation research. Proceedings of the 8th International Congress of Radiation

Research, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis, London, 2, 296-305, 1987.

Elkind, M. M. and H. Sutton, "X-Ray Damage and Recovery in Mammalian Cells in Culture." Nature 184, 1293-1295, 1959.

Elkind, M. M., C. K. Hill, and A. Han, "Repair and Misrepair in Radiation-Induced Neoplastic Transformation." In: Carcinogenesis, eds., E. Huberman and S. H. Barr, Raven Press, New York, 10, 317-336, 1985.

Fearon, E. R., K. R. Cho, J. M. Nigro, S. Kern, J. Simons, J. Ruppert, S. Hamilton, A. Preisinger, G. Thomas, K. Kinzler, and B. Vogelstein, "Identification of a Chromosome 18q Gene That Is Altered in Colorectal Cancer." Science 247, 49-56, 1990.

Feinberg, A. P., D. J. Law, D. Lefrancosi, G. Thomas, "Multistep Genetic Model of Human Colorectal Cancer," Cancer Cells: Molecular Diagnosis of Human Cancer, Cold Spring Harbor Laboratory, New York, pp 245-248, 1989.

Gardner, M. J., M. P. Snee, A. J. Hall, C. A. Powell, S. Downes, and J. D. Terrell, "Results of Case-Control Study of Leukemia and Lymphoma Among Young People Near Sellafield Nuclear Plant in West Cumbria." British Medical Journal 300, 423-429, 1990.

Gillett, N. A., B. L. Stegelmeier, P. J. Haley, and F. F. Hahn, "Epidermal Growth Factor Receptor Expression in Lung Tumors in Beagle Dogs." In: Inhalation Toxicology Research Institute Annual Report, eds., W. E. Bechtold and K. J. Nikula, Albuquerque, New Mexico, December 1989.

Glaser, T., C. Jones, E.C. Douglass, D. Housman, "Constitutional and Somatic Mutations of Chromosome 11p in Wilms' Tumor," Cancer Cells: Molecular and Diagnostics of Human Cancer, Cold Spring Harbor Laboratory, New York, pp 253-277, 1989.

Gray, L. H., "Radiation Biology and Cancer." In: Cellular Radiation Biology, Williams and Wilkins, Baltimore, 7-25, 1965.

Griffen, J. D., A. Rambaldi, E. Vellenga, D. C. Young, D. Ostapovicz, and S. A. Cannistra, "Secretion of Interleukin-1 by Acute Myeloblastic Leukaemic Cells *in Vitro* Induces Endothelial Cells to Secrete Colony Stimulating Factors." Blood 70, 1218-1221, 1987.

Grosovsky, A. J., J. G. de Boer, P. J. de Jong, E. A. Drobetsky, and B. W. Glickman, "Base Substitutions, Frameshifts, and Small Deletions Constitute Ionizing Radiation-Induced Point Mutations in Mammalian Cells." Proceedings of the National Academy of Sciences USA 85, 185-188, 1988.

Grosovsky, A. J., E. A. Drobetsky, P. J. de Jong, and B. W. Glickman, "Southern Analysis of Genomic Alterations in Gamma-Ray-Induced APRT⁻ Hamster Cell Mutants." Genetics 115, 405-415, 1986.

Guerrero, I., P. Calzada, A. Mayer, and A. Pellicer, "A Molecular Approach to Leukemogenesis: Mouse Lymphomas Contain an Activated c-ras Oncogene." Proceedings of the National Academy of Sciences USA 81, 202-205, 1984a.

- Guerrero, I., A. Villasante, V. Corces, and A. Pellicer, "Activation of a c-K-ras Oncogene by Somatic Mutation in Mouse Lymphomas Induced by Gamma Radiation." Science 225, 1159-1162, 1984b.
- Han, A., and M. M. Elkind, "Transformation of Mouse C3H/10T1/2 Cells by Single and Fractionated Doses of X-Rays and Fission-Spectrum Neutrons." Cancer Research 39, 123-130, 1979.
- Han, A., C. K. Hill, and M. M. Elkind, "Repair of Cell Killing and Neoplastic Transformation at Reduced Dose Rates of ^{60}Co γ -Rays." Cancer Research 37, 3328-3332, 1980.
- Hanahan, D., "Transgenic Mice as Probes into Complex Systems." Science 246, 1265-1274, 1989.
- Haran-Ghera, N., "Radiation-Induced Deletion of Chromosome 2 in Myeloid Leukemogenesis." Current Topics in Microbiology and Immunology 149, 35-41, 1989.
- Hayday, A. C., S. G. Gilles, H. Saito, C. Wood, K. Wiman, W. S. Hayward, and S. Tonegawa, "Activation of translocated human c-myc gene by an enhancer in the immunoglobulin heavy chain locus." Nature 307, 334-336, 1984.
- Hieber, L., G. Ponsel, H. Roos, S. Fenn, E. Fromke, and A. M. Kellerer, "Absence of a Dose-Rate Effect in the Transformation of C3H 10T1/2 Cells by Alpha-Particles." International Journal of Radiation Biology 52, 859-869, 1987.
- Hill, C. K., A. Han, F. Buonaguro, and M. M. Elkind, "Multifractionation of ^{60}Co Gamma-Rays Reduces Neoplastic Transformation *in Vitro*." Carcinogenesis 5, 193-197, 1984.
- Hill, C. K., F. M. Buonaguro, C. P. Myers, A. Han, and M. M. Elkind, "Fission-Spectrum Neutrons at Reduced Dose Rates Enhance Neoplastic Transformation." Nature 298, 67-69, 1982.
- Hill, C. K., A. Han, and M. M. Elkind, "Promotion., Dose Rate, and Repair Processes in Radiation-Induced Neoplastic Transformation." Radiation Research 109, 347-351, 1987.
- Hill, C. K., A. Han, and M. M. Elkind, "Promoter-Enhanced Neoplastic Transformation after γ -Ray Exposure at 10 cGy/Day." Radiation Research 119, 348-355, 1989.
- Hill, C. K., B. A. Carnes, A. Han, and M. M. Elkind, "Neoplastic Transformation is Enhanced by Multiple Low Doses of Fission-Spectrum Neutrons." Radiation Research 102, 404-410, 1985.
- Hill, C. K., A. Han, and M. M. Elkind, "Fission-Spectrum Neutrons at a Low Dose Rate Enhance Neoplastic Transformation in the Linear, Low-Dose Region (0-10 cGy)." International Journal of Radiation Biology 46, 11-16, 1984.
- Hubbs, A. F., F. F. Hahn, and D. G. Thomassen, "Altered *In Vitro* Growth Characteristics of Canine Tracheal Epithelial Cells Following Exposure to N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG)." The Toxicologist 8, 160, 1988 (Abstract).

- Jones, C. A., B. A. Sedita, C. K. Hill, and M. M. Elkind, "Influence of Dose Rate on the Transformation of Syrian Hamster Embryo Cells by Fission-Spectrum Neutrons." In: Low Dose Radiation, eds., K. F. Baverstock and J. W. Stather, Taylor & Francis, London, pp 539-546, 1989.
- Kasid, U., A. Pfeifer, R.R. Weichselbaum, A. Dritschilo, and G.E. Mark, "The *raf* Oncogene Is Associated with a Radiation-Resistant Human Laryngeal Cancer." Science 237, 1039-1041, 1987.
- Kasid, U., A. Pfeifer, T. Brennan, M. Beckett, R. R. Weichselbaum, A. Dritschillo, and G. E. Mark, "Effect of Antisense *c-raf-1* on Tumorigenicity and Radiation Sensitivity of a Human Squamous Carcinoma." Science 243, 1354-1356, 1989.
- Klein, G. "In defense of the "old" Burkitt lymphoma scenario." Advances in Viral Oncology 1, 207-210, 1987.
- Knudson, A. G., "Retinoblastoma: a Prototypic Hereditary Neoplasm." Seminars in Oncology 5, 57-60, 1978.
- Kucherlapati, R., ed., Gene Transfer, Plenum Press, New York, 1986.
- Kwok, T. T. and R. M. Sutherland, "Enhancement of Sensitivity of Human Squamous Carcinoma Cells to Radiation by Epidermal Growth Factor." Journal of the National Cancer Institute 81, 1020-1024, 1989.
- Lambert, M. and C. Borek, "X-Ray-Induced Changes in Gene Expression in Normal and Oncogene-Transformed Rat Cell Lines." Journal of the National Cancer Institute 80, 1492-1497, 1988.
- Land, H., L. F. Parada, and R. A. Weinberg, "Tumorigenic Conversion of Primary Embryo Fibroblasts Requires at Least Two Cooperating Oncogenes." Nature 304, 596-602, 1983.
- LeBeau, M. M. and Rowley, J. D., "Chromosomal Abnormalities in Leukemia and Lymphoma: Chemical and Biological Significance. In: Advances in Human Genetics, Vol. 15 eds., H. Harris and K. Hirschhorn, Plenum Press, pp. 1-54, 1986.
- Leder, A., Pattengale, P.K., Kuo, A., Stewart, T.A. and Leder, P. "Consequences of widespread deregulation of the *c-myc* gene in transgenic mice: Multiple neoplasms and normal development." Cell 45, 485-490, 1988.
- Liber, H. L. and W. G. Thilly, "Mutation Assay at the Thymidine Kinase Locus in Diploid Human Lymphoblasts." Mutation Research 94, 467-485, 1982.
- Liber, H. L., K. M. Call, and J. B. Little, "Molecular and Biochemical Analyses of Spontaneous and X-Ray-Induced Mutants in Human Lymphoblastoid Cells." Mutation Research 178, 143-153, 1987.
- Liskay, R. M., J. L. Stachelek, and A. Letsou, "Homologous Recombination between Repeated Chromosomal Sequences in Mouse Cells." Cold Spring Harbor Symposium in Quantitative Biology 49, 183-189, 1984.

- Little, J. B., "Quantitative Studies of Radiation with the A31-11 Mouse BALB/3T3 cell line." Cancer Research 39, 1474-1480, 1979.
- Malkinson, A. M., "The Genetic Basis of Susceptibility to Lung Tumors in Mice." Toxicology 54, 241-271, 1989.
- Matrisian, L. M., G. T. Bowden, P. Krieg, G. Furstenberger, J. P. Briand, P. Leroy, and D. Breathnach, "The mRNA Coding for the Secreted Protease Transin is Expressed More Abundantly in Malignant than in Benign Tumors." Proceedings of the National Academy of Sciences, USA 83, 9413-9417 (1986).
- Metalli, P., V. Covelli, and G. Silini, "Dose-Incidence Relationships of Reticulum Cell Sarcoma in Mice." In: Late Effects of Ionizing Radiation (IAEA-SM-224/406) 11, 341-349, 1978.
- Miller, R., and E. J. Hall, "Effect of X-Ray Dose Fractionation on the Induction of Oncogenic Transformation *in Vitro* in C3H/10T1/2 Mouse Embryo Cells." Nature 72, 58-60, 1978.
- Moore, M., D. M. DeMarini, F. J. De Serres, K. R. Tindall, eds., Mammalian Cell Mutagenesis, Banbury Report No. 28, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1987.
- Müller, W. A., W. Gössner, O. Hug, and A. Luz, "Late Effects after Incorporation of the Short-Lived α -Emitters ^{224}Ra and ^{227}Th in Mice." Health Physics 35, 33-35, 1978.
- Newcomb, E. W., J. J. Steinberg, and A. Pellicer, "ras Oncogenes and Phenotypic Staging in N-Methylnitrosourea- and γ -Irradiation-Induced Thymic Lymphoma in C57Bl/6J Mice." Cancer Research 49, 5514-5521, 1988.
- Nomura, T., "Parental Exposure to X-Rays and Chemicals Induces Heritable Tumors and Anomalies in Mice." Nature 296, 575-577, 1982.
- Nussenzweig, M. C., E. V. Schmidt, A. C. Shaw, E. Sinn, J. Campos-Torres, B. M. Mathey-Prevot, P. K. Pattengale, and P. Leder, "A Human Immunoglobulin Gene Reduces the Incidence of Lymphomas in c-myc Bearing Transgenic Mice." Nature 336, 446-450, 1988.
- Parker, R. D., C. A. Waldren, T. Hei, D. Wong, and T. T. Puck, "Analysis of Mutation Frequency Curves and Survival Curves Applied to the A₁ Hybrid Cell System." Journal of Theoretical Biology 132, 113-117, 1988.
- Pattengale, P. K., T. A. Stewart, A. Leder, E. Sinn, W. Muller, I. Tepler, E. Schmidt, and P. Leder, "Pathology and Molecular Biology of Spontaneous Neoplasms Occurring in Transgenic Mice Carrying and Expressing Activated Cellular Oncogenes." American Journal of Pathology 135, 39-61, 1989.
- Pattengale, P. K., and C. R. Taylor, "Experimental Models of Lymphoproliferative Disease, Mouse as a Model for Human Non-Hodgkin's Lymphomas and Related Leukemias." American Journal of Pathology 113, 237-265, 1983.
- Ponder, B., "Cancer, Gene Losses in Human Tumors," Nature 335, 400-402, 1988.

- Puck, T. T., "Consideration of Measurements and Calculations Involved in Assessment of Ionizing Radiation and Other Mutagens." Letters in Mathematical Physics 10, 225-230, 1985.
- Ramakrishnan, N., S. M. Chiu, and N. L. Oleinick, "Yield of DNA-Protein Cross-Links in γ -Irradiated Chinese Hamster Cells." Cancer Research 47, 2032-2035, 1987.
- Redpath, J. L., C. Sun, M. Colman, and E. J. Stanbridge, "Neoplastic Transformation of Human Hybrid Cells by Gamma Irradiation: A Quantitative Assay." Radiation Research 110, 468-472, 1987.
- Reznikoff, C.A., D. W. Brankow, and C. Heidelberger, "Establishment and Characterization of a Cloned Line of C3H Embryo Cells Sensitive to Post Confluence Inhibition of Division." Cancer Research 33, 3231-3238, 1973.
- Sager, R., "Tumor Suppressor Genes: The Puzzle and the Promise." Science 246, 1406-1412, 1989
- Sawey, M. J., A. T. Hood, F. J. Burns, and S. J. Garte, "Activation of c-myc and c-K-ras Oncogenes in Primary Rat Tumors Induced by Ionizing Radiation." Molecular and Cellular Biology 7, 932-935, 1987.
- Saxon, P. J., E. S. Srivatsan, and E. J. Stanbridge, "Introduction of Human Chromosome 11 via Microcell Transfer Controls Tumorigenic Expression of HeLa Cells." The EMBO Journal 5, 3461-3466, 1986.
- Shtivelman, E., B. Lifshitz, R. P. Gale, and E. Cananni, "Fused transcript of *abl* and *bcr* genes in chronic myelogenous leukemia." Nature 315, 550-554, 1985.
- Silver, A. R. J., G. Breckon, W.K. Masson, J. Adam, J. Boulwood, and R. Cox, "Radiation Induced Chromosome 2 Rearrangement and Initiation of Murine Acute Myeloid Leukemia." Radiation Research (in press), 1990.
- Silver, A. R. J., G. Breckon, W.K. Masson, J. Adam, A. R. Shaw, and R. Cox, "Interleukin-1 β Gene Dereglulation Associated with Chromosomal Rearrangement: A Candidate Initiating Event for Murine Radiation-Myeloid Leukemogenesis?" Molecular Carcinogenesis (in press), 1990.
- Silver, A. R. J., G. Breckon, W.K. Masson, D. Malowany, and R. Cox, "Studies on Radiation Myeloid Leukaemogenesis in the Mouse." In: Radiation Research. Proceedings of the 8th International Congress of Radiation Research, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis, London, 2, 494-500, 1987.
- Sklar, M. D., "The *ras* Oncogenes Increase the Intrinsic Resistance of NIH 3T3 Cells to Ionizing Radiation. Science 239, 645-647, 1988.
- Smith, P. G., and R. Doll, "Mortality Among Patients with Ankylosing Spondylitis after a Single Treatment Course with X-Rays." British Medical Journal 284, 449-460, 1982.
- Spiess, H. and C. W. Mays, "Protraction Effect on Bone-Sarcoma Induction of Radium-224 in Children and Adults." In: Radionuclide Carcinogenesis, eds.,

C. L. Saunders, et al., USAEC Symposium Series 29 (CONF-720505), National Technical Information Service, Springfield, Virginia, pp 437-450, 1973.

Stamato, T. D., L. Hinkle, A. Collins, C. Waldren, "Chinese Hamster Ovary Mutant UV-1 is Hypomutable and Defective in a Postreplication Recovery Process." Somatic Cell and Molecular Genetics 7, 307-320, 1981.

Stegemeier, B. L., N. A. Gillett, G. Kelly, A. H. Rebar, and F. F. Hahn, "Oncogene Expression of Radiation-Induced Proliferative Epithelial Foci in the Lungs of Rats." In: Inhalation Toxicology Research Institute Annual Report, eds., W. E. Bechtold and K. J. Nikula, Albuquerque, New Mexico, December 1989.

Sugawara, O., M. Oshimura, M. Koi, L.A. Annab, and J. C. Barrett, "Induction of Cellular Senescence in Immortalized Cells by Human Chromosome 1." Science 247, 707-710, 1990.

Sun, C., J. L. Redpath, M. Colman, and E. J. Stanbridge, "Further Studies on the Radiation-Induced Expression of a Tumor-Specific Antigen in Human Cell Hybrids." Radiation Research 114, 84-93, 1988.

Takahashi, T., M. N. Nau, I. Chiba, M. J. Birrer, R. K. Rosenberg, M. Vinocoor, M. Leavitt, H. Pass, A. F. Gazdar, and J. D. Minna, "p53: A Frequent Target for Genetic Abnormalities in Lung Cancer." Science 246, 491-494, 1989.

Terzaghi, M., A. Klein-Szanto, and P. Nettesheim, "Effect of the Promoter 12-O-tetradecanoylphorbol-13-acetate on the Evolution of Carcinogen-altered Cell Populations in Tracheas Initiated with 7,12-dimethylbenz(a)anthracene." Cancer Research 43, 1461-1466, 1983.

Terzaghi, M., P. Nettesheim, and L. Riester, "Effect of Carcinogen Dose on the Dynamics of Neoplastic Development in Rat Tracheal Epithelium." Cancer Research 42, 4511-4518, 1982.

Terzaghi-Howe, M., "Induction of Preneoplastic Alterations by X-Rays and Neutrons in Exposed Rat Tracheas and Isolated Tracheal Epithelial Cells." Radiation Research 120, 352-363, 1989.

Thacker, J. "The Molecular Nature of Mutations in Cultured Mammalian Cells: A Review." Mutation Research 150, 431-442, 1985.

Thomson, J. F., F. S. Williamson, and D. Grahn, "Life Shortening in Mice Exposed to Fission Neutrons and γ rays. VI. Studies with the White-Footed Mouse, *Peromyscus leucopus*." Radiation Research 108, 176-188, 1986.

Ullrich, R. L., "Tumor Induction in BALB/c Mice after Fractionated or Protracted Exposure to Fission-Spectrum Neutrons." Radiation Research 97, 587-597, 1984.

Ullrich, R. L., "The Rate of Progression of Radiation-Transformed Mammary Epithelial Cells after Low-Dose-Rate Neutron Irradiation." Radiation Research 105, 68-75, 1986.

- Ullrich, R. L., M. C. Jernigan, G. E. Cosgrove, L. C. Satterfield, N. D. Bowles, and J. B. Storer, "The Influence of Dose and Dose Rate on the Incidence of Neoplastic Disease in RFM Mice after Neutron Irradiation." Radiation Research 68, 115-131, 1976.
- Ullrich, R. L. and J. B. Storer, "Influence of γ Irradiation on the Development of Neoplastic Disease in Mice. III. Dose Rate Effects." Radiation Research 80, 325-342, 1979.
- Upton, A. C., "Evolving Perspectives on the Concept of Dose in Radiobiology and Radiation Protection." Health Physics 44, 605-614, 1988.
- Upton, A. C., M. L. Randolph, and J. W. Conklin, "Late effects of Fast Neutrons and Gamma-Rays in Mice as Influenced by the Dose Rate of Irradiation: Induction of Neoplasia." Radiation Research 41, 467-491, 1970.
- Urlaub, G., P. J. Mitchell, E. Kas, L. A. Chasin, V. L. Furnanage, T. M. Myoda, and J. Hamlin, "Effects of Gamma Rays at the Dihydrofolate Reductase Locus: Deletions and Inversions." Somatic Cell Molecular Genetics 12, 555-566, 1986.
- van de Vijver, M., R. van de Berselaar, R. Devilee, P. Cornelisse, C. Peterse, and J. Nusse, "Amplification of the c-neu (c-erbB-2) Oncogene in Human Mammary Tumors is Relatively Frequent and Is often Accompanied by Amplification of the Linked c-erbA Oncogene." Molecular and Cellular Biology 7, 2019-2023, 1987.
- Vogelstein, B., E. R. Fearon, S. J. Baker, J. M. Nigro, S. E. Kern, S. R. Hamilton, J. Bos, M. Leppert, Y. Nakamura, and R. White, "Genetic Alterations Accumulate During Colorectal Tumorigenesis." In: Recessive Oncogenes and Tumor, eds., W. Cavenee, N. Hastie, and E. Stanbridge, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 73-80, 1989.
- Waldren, C., L. Correll, M. A. Sognier, and T. T. Puck, "Measurement of Low Levels of X-Ray Mutagenesis in Relation to Human Disease." Proceedings of the National Academy of Sciences USA 83, 4839-4843, 1986.
- Waldren, C., C. Jones, and T. T. Puck, "Measurement of Mutagenesis in Mammalian Cells." Proceedings of the National Academy of Sciences USA 76, 1358-1362, 1979.
- Wang, Y., V. M. Maher, R. M. Liskay, and J. J. McCormick, "Carcinogens Can Induce Homologous Recombination between Duplicated Chromosomal Sequences in Mouse L Cells." Molecular and Cellular Biology 8, 196-202, 1988.
- Wang-Peng, J., "3p Deletion and Small Cell Lung Cancer," Mayo Clinic Proceedings 64, 256-260, 1989.
- Weston, A., J. C. Wiley, R. Modali, H. Sugimura, C. M. McDowell, J. Rasau, D. Light, A. Haugen, D. L. Mann, D. F. Trump, et al., "Differential DNA Sequence Deletions from Chromosomes 3, 11, 13, and 17 in Squamous-Cell Carcinoma, Large-Cell Carcinoma, and Adenocarcinoma of the Lung," Proceeding of the National Academy of Science USA 86, 5099-5103, 1989.

- Watanabe, M., N. Suzuki, S. Swada, and O. Nokaïdo, "Repair of Lethal, Mutagenic and Transforming Damage Induced by X-rays in Golden Hamster Embryo Cells." Carcinogenesis 5, 1293-1299, 1984.
- White, R., "High-Resolution Mapping of Inherited Tumor Genes." In: Recessive Oncogenes and Tumor, eds., W. Cavenee, N. Hastie, and E. Stanbridge, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 27-31, 1989.
- Yandell, D. W., T. P. Dryja, and J. B. Little, "Somatic Mutations at a Heterozygous Autosomal Locus in Human Cells Occurs More Frequently by Allele Loss than by Intragenic Structural Alterations." Somatic Cell Molecular Genetics 12, 255-263, 1986.
- Yang, T. C. H., L. M. Craise, M. T. Mei, and C. A. Tobias, "Dose Protraction Studies with Low- and High-LET Radiations on Neoplastic Cell Transformation *in Vitro*." Life Science in Space Research 22, 137-147, 1986.
- Yunis, J. J., C. D. Bloomfield, and K. M. Engrud, "All Patients with Acute Nonlymphocytic Leukemia May Have a Chromosomal Defect." New England Journal of Medicine 305, 135-139, 1981.

Task 4

"Methods, Techniques, and Instruments for Mechanistic Studies"

4.1 INTRODUCTION

To undertake a research program of the mechanisms of radiation-induced neoplasia, a number of techniques would be needed. The experimental methods to be used would have to be comprehensive, in view of the objectives and scope of the study, and would include a gamut of measurements made with material from molecules and cells *in vitro* to tissues and organs *in vivo*.

The various research techniques to be discussed will, in many instances, employ experimental systems derived from rodent material. It is important to establish, therefore, what similarities and/or differences exist between rodent and human systems in order to validate the extrapolation of results with the former to estimates of risk relevant to the latter.

Wherever possible, the experimental methods and materials to be employed should be based upon techniques that have sufficient sensitivity and resolution to permit useful measurements, of the various endpoints of interest, in the region of low doses, i.e., < 10 cSv, delivered at high and low dose rates. The measurements would apply to molecular and cellular endpoints, and, as required, effects on tissues and organs in animals. However, in instances where the foregoing is not immediately achievable, efforts should be made to determine the linear component of the dose dependence at higher doses, and whether or not it may depend on dose rate, or dose protraction (see Elkind, 1977; Elkind et al., 1985). As experimental systems are used, efforts should be made to improve the methods sufficiently to permit reliable extensions of the measurements into the low-dose region. Thus, if the dose dependence were shown to be linear, extrapolation from higher to zero dose could permit estimates to be made of the degree of effect corresponding to low doses.

This Task consists largely of a discussion of the techniques, which would be needed in a molecularly-based radiation research program, plus descriptive information complete enough to permit their strengths and weaknesses to be identified. As required, the need for new methods is indicated.

4.2 HUMAN POPULATIONS

As its principal objective, the research program would be directed at increasing the understanding of how ionizing radiation produces neoplasia in people so as to reduce the existing uncertainty in estimates of risk due to low doses which may be delivered over extended periods (see BEIR V Report, 1990). The epidemiological data that already exist make clear that a number of quantitatively different dose and dose rate dependencies will be involved. Such differences might be expected from the knowledge that the principal unifying feature of all cancers--unregulated, physiologically abnormal growth incompatible with life--can be arrived at in a variety of ways (see Sections 2.3 & 2.5; BEIR V Report, 1990). Not only are radiobiological parameters--i.e., dose, dose rate, radiation quality, and so on--relevant to the induction process, but also genetic parameters that may characterize subgroups of people. Among the latter are various repair deficiencies, immunological abnormalities, familial susceptibilities, and the like. The importance of variability in the population

at large is typified by inherited genetic anomalies, including human repair-deficiency disorders, that are associated with high incidences of spontaneous neoplasms such as ataxia telangiectasia, xeroderma pigmentosum, Fanconi's anemia, and others.

4.2.1 The "Boundary Conditions" of Neoplasia

Although histologically and pathologically a radiation-induced cancer is not distinguishable from one that arises spontaneously, or is induced by another etiological agent, epidemiological studies have shown that some neoplasms, broadly speaking, are radiation-inducible, that is *radiogenic*, and others appear not to be. From this fact alone, it follows that radiobiological formulations of risk vs. dose must be neoplasm specific to be consistent with epidemiological data. In addition, epidemiological studies may be instrumental in identifying genetic, life-style, and environmental factors that influence tumorigenic yields from which important leads about mechanisms may be derived. Hence, within the uncertainties that exist in epidemiological data, a radiation research program should be constrained by the "boundary condition" that are represented by observations made in human populations under the particular radiobiological conditions that may have applied. In addition, radiobiologists and epidemiologists must share their insights in the interests of maximizing progress.

4.3 MOLECULAR TECHNIQUES FOR STUDIES OF DNA

Virtually all the heritable information in a cell is in its genomic DNA so that changes (mutations) in the information content of the genome potentially can have very large biological effects. In addition, the molecules of DNA in the nucleus are the largest and most important molecular species in a cell constituting, therefore, the largest and potentially the most important radiation target in a cell. For these reasons, a number of molecular techniques have been developed to measure the induction and repair of different kinds of lesions in DNA. Many of these techniques are well established and have been fully described. Hence, they will be referred to only briefly.

4.3.1 Measurement of Scissions in DNA

One of the most important advances in characterizing large mutations, which result from double-strand breaks in DNA, comes from the application of the technique of pulsed field gel electrophoresis (PFGE). The strength of this method for radiobiology lies in its ability to resolve fragments of DNA ranging from a few Kbp (kilo base pairs) to those that are very large, over 10 Mbp (mega base pairs; see Lai et al., 1989), so that effects of radiation on molecules approaching the size of those found in a cell can be examined. Consequently, it should be possible to measure discontinuities in DNA produced by low doses.

Although the principle of this technique is not fully understood, it is believed that when an electric field is applied to a gel containing DNA, the DNA elongates and migrates in the direction of the field. If, after a period of time, the first electric field is replaced by a second one applied at an angle to the first, the DNA changes its conformation and become reorientated to migrate in the new direction. The time required for this reorientation is a sensitive function of molecular weight. Because larger molecules reorient more slowly than smaller ones, the latter migrate further into the gel than the former.

The PFGE technique has been employed to isolate large fragments of the genes of mammalian cells in order that their break points may be analyzed (Gardiner, et al., 1988), and also to provide new ways of studying very large fragments of DNA (Barlow and Lehrach, 1987). The method has been used to provide a measure of the number of double-strand breaks in DNA that are introduced when cells are X-irradiated--e.g., in yeast (Contopoulou et al., 1987), or in mammalian cells (Blocher et al., 1989; Stamato and Denko, 1990; Ager et al., 1990.)--and PFGE also facilitates studies of the repair of such lesions.

DNA need not be extracted from cells before PFGE analysis. After the cells are embedded in the gel, it suffices to lyse the cells--under neutral, nondenaturing conditions to preserve double strandedness--to permit the electrophoretic migration of the DNA. Hence, PFGE is a method of analyzing the size distribution of large pieces of double-stranded DNA that is technically easier to use, and at least as sensitive, as the more traditional methods; that is, estimating the size of double-stranded molecules by sedimentation in neutral sucrose gradients, or by their rate of elution through filters following cell lysis under neutral, nondenaturing conditions. The PFGE method has the added advantage of providing direct estimations of the molecular weights of these fragments by comparing their migration with that of DNA of known size, for example, the DNA from the chromosomes of various yeasts. Further, because PFGE can be applied to alkaline, denaturing gels, it should be applicable to the study of other kinds of lesions--for example, single-strand breaks--that are also known to be produced in DNA by ionizing radiation.

For molecular weights larger than those of the chromosomes of yeast cells (~10 Mbp), which are used as standards of size, the technique of PFGE does not render molecular distributions that are readily interpretable in terms of molecular weight at least because standards larger than yeast chromosomes are not as yet available. Hence, although progress has been made (Ulanovsky et al., 1990), further developments are needed in the theory and practice of the method if it is to be successfully applied to the analysis of the DNA from mammalian cells following low doses of radiation; doses that are expected to produce molecular fragments approaching 1×10^{10} daltons. Still, the PFGE method has already been applied to the study of breakage and repair at doses less than 0.5 Gy.

4.3.2 Changes in the DNA Code by Sequencing

Molecular methods have been developed that facilitate the analysis of changes in DNA caused by various mutagenic agents that permit the study of how these changes are produced. One of these methods, DNA sequencing, provides the exact base sequence of fragments of DNA.

Two methods have been developed for accurately establishing the order of the four bases (adenine, guanine, cytosine, and thymine) along isolated fragments of DNA. The first uses chemical reagents to modify specific bases, interrupting the DNA chain at specific sites, and hence allowing the original base sequence to be inferred (Maxam and Gilbert, 1977). The second, the dideoxy-mediated chain termination method, employs an enzyme, DNA polymerase I, to produce copies of the DNA of random lengths such that, when the fragments are separated by electrophoresis on gels, a direct determination of the order of the nucleotides can be made (Sanger et al., 1977). Although only 200 or so nucleotides can be sequenced in a given gel, by the use of multiple gels the linear order of several

thousand of bases is possible. The dideoxy method is now the one that is most widely employed.

In principle, sequencing methodologies could be used to analyze fragments of genes produced by the exposure of cells to ionizing radiation, after the fragments have been separated via PFGE for example, in order to determine changes in base sequences that may have been produced by the radiation exposure. However, because the size of the fragments that can be analyzed is limited, from about 100 to a few 1000 base pairs, ordinarily very large doses would have to be involved. Consequently, improvements in the methods are needed to enable DNA sequence analysis to be useful in the range of doses that is physiologically and radiobiologically meaningful. The rate of progress in sequencing the human genome depends significantly on the size of the DNA fragments that can be analyzed. Hence, technical developments for that project may have application to radiation studies. For special purposes, the sequencing techniques as currently applied can provide useful information, for example, in analyzing mutations produced in specific genes due to changes in sequences at junctions between exons and introns.

4.3.3 Application of the Polymerase Chain Reaction

The polymerase chain reaction (PCR) provides a means of synthesizing enough DNA for chemical/molecular analysis, without resorting to amplification of a DNA-containing vector in a bacterium (see Section 3.3.2.1), even when only a single copy of the molecule in a single cell may be available.

The PCR technique ordinarily requires a knowledge, *a priori*, of the DNA sequence on either side of a locus of interest for the method to be applied successfully. As usually applied, the technique employs the initial and terminal sequences, serving as primers, biochemically to synthesize the full length of the molecule from both complementary strands of DNA including the locus of interest. A series of heating and cooling steps then is used to denature the double-stranded DNA that was synthesized, to allow for the association of a DNA polymerase I with the denatured single strands, and for the synthesis again of the molecule of interest. Repeated cycles of the foregoing result in an exponential increase in the number of molecules because, at each repeat, the number is doubled. This technique appears to have considerable potential for the study of a given segment of DNA, at the level of its base composition (Mullis et al., 1986), with a precision heretofore not possible to achieve.

Although relatively large molecules of DNA can be analyzed, as with the methods of DNA sequencing, the PCR technique currently is limited to molecular sizes that would require large doses of radiation to be hit. Advances are needed to reduce the practical limitations on fragment size that are now analyzable by the PCR method if it is to become applicable to the study of changes due to small-to-moderate doses. Further, in the usual application of the method, only changes within the region between the primers can be analyzed. Strategies have been proposed for PCR amplification when only one priming site remains, a procedure that should permit the assessment of extragenic deletions.

4.3.4 Detection and Characterization of Molecular Additions to DNA

A number of methods have been developed to examine the adducts formed in DNA, i.e., molecules that become covalently bound to DNA, due to the exposure of cells to environmental agents, such as ultraviolet light, and to certain

chemicals (see Bridges et al., 1982; Roza et al., 1988). The value of these techniques lies principally in their sensitivity for the detection of damage to bases due to additions.

Among the techniques for analyzing the formation of adducts in DNA are fluorescence, high-performance liquid chromatography, and high-affinity monoclonal antibodies that are specific for various DNA lesions including damaged bases. Another sensitive method of detecting base damage is the ^{32}P -postlabeling assay devised by Randerath and his colleagues (Randerath et al., 1981). A single adduct among millions of normal bases can be detected by the ^{32}P -postlabeling method.

The value of these technologies for radiation studies remains to be determined. The spectrum of adducts that are induced by radiation is not known as yet. In addition to the attachment of relatively small molecules to DNA, it is known that radiation produces DNA-protein crosslinks the properties of which are poorly understood. Hence, improvements in current techniques, as well as new approaches, are needed that will enable both the qualitative and quantitative examination of radiation-induced additions to DNA or damage to specific bases.

4.4 CELLS AND CELL SYSTEMS IN VITRO

The developments of the past 50 years in the technology of cultivating mammalian cells have been responsible for many of the insights that have been realized in cancer research as well as in biology as a whole. Still, improvements are needed if this technology is to fulfill its full potential usefulness.

In principle, what would be optimal is to have cells in culture whose properties are the same as those of the tissue and host of origin. The tissues of interest should be those that are of primary concern because they are susceptible to radiation-induced neoplasms, and the hosts should be those that would form a logical sequence in extrapolating radiobiological data from rodents-to-people. At the present, this situation does not exist, as indicated below.

4.4.1 Requirements for Optimal Cell Cultivation

To assess accurately the radiobiological and other properties of cells in culture, metabolic and physiological conditions, should be developed which simulate those that apply in the host. Simply to establish conditions *in vitro* that may enable cells to live (i.e., to metabolize, respire, maintain morphological integrity, etc.) and, in addition, may permit cell growth and division, does not insure that the responses to radiation, or to other inducers of neoplastic transformation, will be close enough to what they would be *in situ* to yield valid results.

A knowledge of the optimal growth conditions for the cultivation of mammalian cells from the tissues and species of principal interest is needed. Specific substrate and growth-factor requirements have to be worked out which, in addition to the composition of the basal medium, permit stable cultivation. Because various sera frequently constitute 5-15 percent of culture media, and because the properties and composition of sera have not been fully determined and may fluctuate from batch-to-batch, it would be desirable to develop media for

cell cultivation that are fully defined as well as representative of the growth conditions in the host.

4.4.2 Types of Cells

Although neoplasms of all developmental types of tissue are known to occur, human tumors arise most frequently in epithelium. In general, normal epithelial cells have been more difficult to culture than fibroblasts. As a result, most of the mammalian cell lines that are available for study were derived from fibroblasts. For quantitative radiobiological studies, the need exists for a greater diversity of cell types and cultivation systems. Further, cell strains and lines derived from tissues of primary interest should be developed including lung, mammary gland, colorectal, and thyroid gland epithelium as well as parenchymal cells of the blood-forming system.

4.4.3 Systems for the Analysis of Somatic Mutations

Despite a large amount of effort, the mechanisms underlying mutagenesis from ionizing radiation are less well understood than for ultraviolet light and many chemicals, particularly alkylating compounds. A principal reason for this disparity is that, in most test systems, the mutagenic action of ionizing radiation relative to its lethal action is low, making the analysis of mutagenesis more difficult.

Somatic mutation is defined as any change in the composition of the DNA of a somatic cell that can be inherited by its progeny (Section 3.3.2). For convenience in discussion, mutations are divided according to size into intragenic mutations (i.e., changes within a single gene), as opposed to extragenic, multilocus, or chromosomal mutations. The latter group consist of changes to large portions of a chromosome including translocations, insertions, multilocus deletions, and the loss or gain of an entire chromosome.

4.4.3.1 Shuttle Vectors

Many innovative methods have been developed that combine genetic and recombinant DNA technologies to quantitate mutation induction and to shed light on the mechanism involved. Among the most popular of these technologies are those based upon the use of a "shuttle vector." (See Section 3.3.2.1.)

A shuttle vector is a DNA sequence containing a well-defined bacterial or viral gene and the control elements that allow the vector to be replicated as an episomal particle, a plasmid, in a bacterium or in a mammalian cell (see Calos, 1986). Physically, plasmids are small circles of DNA, or minichromosomes, that occur naturally in bacteria and in some other micro-organisms. Although plasmids can be replicated independently of the host-cell genome, in some instances they become incorporated into the host's chromosomes.

Some plasmids can be engineered so that they can be grown and studied both in a bacterium and a mammalian cell. That is, they can be made to "shuttle" from one host to the other. The marker gene in the plasmid can be exposed to a mutagen, such as ionizing radiation, either extracellularly as purified DNA, or while the plasmid is in the mammalian cell. In the latter circumstance, the plasmid then can be retrieved, amplified by inserting it into a suitable bacterium, or via the polymerase chain reaction, and then subjected to analysis

by the technique of *restriction-fragment-length polymorphism*, or by sequencing of the DNA (Maxam and Gilbert, 1977; Sanger et al., 1977).

A plasmid that contains a gene called *supF*, as a target for mutation, has been used in several studies. Generally the plasmid would have been irradiated extracellularly and then introduced into a mammalian cell via DNA transfection. The plasmid would not have propagated continuously; rather, it would have been retrieved after about 48 hours and used to transform *E.coli* bacteria (Bredberg, et al., 1986).

Although this system has been used successfully to analyze mutations caused by chemical agents and by ultraviolet light, it works very poorly for ionizing radiation because the target size of the gene is very small (203 bp) so that mutations in DNA larger than this escape detection. The high background level of mutation in this plasmid also presents a serious problem.

Other studies based on shuttle-vector technology have used the 1000 bp *lacI* gene of *E.coli* as a target (Calos, 1986). The most useful of the latter vectors for studies with radiation would seem to be the plasmid pSVi4 *lacI* which, unlike similar constructs, is flanked by important sequences only on one side so that deletions can extend into the neighboring gene and not be lethal. This vector is capable of revealing deletions in the range of a few thousand base pairs and, with further development, it could be used for mechanistic studies. The vector has the disadvantage, however, of a relatively high spontaneous mutation frequency, about 3.5×10^{-4} , which is well above that to be expected for a mammalian gene, so that caution is required in extrapolating results obtained with this vector to mammalian cells.

Other shuttle vector systems have been devised that can be grown in a variety of mammalian-cell hosts. Among these are the pSV5gpt *lacI*, which contains the origin of replication of polyoma virus, so that it can replicate in mouse, Syrian hamster, and certain other rodent cell lines. Still other plasmids have been based on the bovine papillomavirus, to permit the plasmids to grow in a variety of mammalian cells, but they have proved to have high levels of spontaneous mutations making them unsuitable for studies with ionizing radiation.

Vectors based on the human herpes Epstein-Barr virus (EBV) appear promising for studies of the mechanism of the production of larger mutations. These vectors contain the viral signals that are needed for its replication in mammalian cells, a gene for hygromycin to enable the selection of those mammalian cells that take up the virus, and portions of the plasmid pBR322 to facilitate the selection of colonies of the bacterium *E. coli* that incorporate the vector and permit its replication. These vectors can be stably carried, at high copy numbers, in a wide range of human and other primate cells so long as selection with hygromycin is maintained. Preliminary reports indicate that the rate of spontaneous mutation in these cells is reasonably low. New constructs based on EBV have the potential of offering a target size perhaps as large as 80% of the entire plasmid, approximately 9 Kb, suggesting that they may facilitate studies of the larger kinds of mutations that predominate in cells exposed to ionizing radiation.

4.4.3.2 Endogenous Genes

Although studies with vectors of various kinds are potentially quite useful, ultimately the understanding that is required is the mechanism of the

mutagenesis of endogenous genes, in particular, in mammalian cells (see Breimer, 1988; Thacker, 1986). Ionizing radiation can induce single base changes as illustrated by the spectrum of point mutations produced in micro-organisms (see Hutchinson, 1989; Das et al., 1986). However, in mammalian cells, extragenic changes predominate (Thacker et al., 1978). Reasonably high relative frequencies of radiation-induced intragenic mutations have been reported only in a minority of instances, for example in reference to the adenine phosphoribosyl transferase (*aprt*) gene (Liber et al., 1986; Grosovsky et al., 1988), or the *ras* oncogene (Guererro et al., 1984).

Most mutations caused by ionizing radiation are not intragenic but rather are frequently large extragenic changes (see Thacker and Cox, 1986; Yandell et al., 1986). The task of molecularly characterizing mutations in endogenous genes in mammalian cells has been aided by the cloning (i.e., by amplifying the number of mutated molecules via the use of an appropriate bacterial system) of the structural sequences (the exons plus the introns), which comprise the entire gene, and the cDNA sequences (the exons only), which code for messenger RNA and, therefore, the protein coded by the gene. (The structural sequence is the entire linear array of nucleotides of a gene whereas the cDNA sequence is the linear array of only the coding part of the gene.) Specific genes whose structural and cDNA sequences are available include *aprt*, hypoxanthine-guanine phosphoribosyl transferase (*hgpert*), dihydrofolate reductase (*dhfr*), and thymidine kinase (*tk*).

The locus most frequently studied for the effects of ionizing radiation has been the *hgpert* gene of Chinese hamster cells and of human lymphocytes. Although the structural gene contains about 40 Kb of DNA, the limit of resolution of mutational changes has been approximately 0.5 kb because of the nature of the cDNA probe that was used (Liber et al., 1987).

Several mutants have been characterized at the *aprt* locus (Liber et al., 1986; Grosovsky et al., 1988), and at the *dhfr* locus (Urlaub et al., 1986). In the latter instance, all of the mutations that were analyzed contained major rearrangements, such as a deletion of the entire 210 Kb sequence or very large inversions. Mutations have also been characterized at the *tk* locus in human lymphoblastoid cells (Yandell et al., 1986), and in mouse lymphoma cells (Evans et al., 1986). A human/Chinese hamster cell hybrid has been used by Waldren and collaborators (1986) to study mutations induced by X-rays and neutrons in genes that code for human cell-surface antigens; a spectrum of mutations were detected from intragenic changes to the loss of the entire chromosome containing the genes for the antigenic determinants.

The delay in the expression of a mutated phenotype (Bradley, 1980; Stamato et al., 1987; Bradley and Laviolette, 1989), and the delayed expression of lethal mutations (Mothersill and Seymour, 1987), are additional genetic phenomena that can influence neoplastic transformation. However, the latter change appears to be one that is significant only after relatively large doses of radiation (Gorgojo and Little, 1989).

Although the genetic markers, and the cell systems that have been developed for studying them, have yielded useful information, in the context of genetic changes that lead to neoplastic transformation, the materials and methods need to be improved.

First, a greater diversity of cell types is needed both in respect to histological types (endothelial, mesenchymal, and epithelial) and to

species (rodent to human). Further, cell lines are needed that permit comparisons between induction frequencies in repair-competent vs. repair-deficient cells.

Second, a larger range of genetic markers are needed. These markers should enable, for comparative purposes in the same cell line, the measurement of intragenic vs. extragenic changes.

Third, more markers are needed in appropriate cell systems that would help to identify mutations other than those connected with the salvage pathways of DNA synthesis. In particular, markers that can be identified with the regulation of cell growth would be appropriate.

And last, more markers are needed in hemizygous and heterozygous configurations to test for the dependence of frequencies of induction on the loss of flanking genes when they are present as single-copies vs. double-copies, respectively. Homozygous configurations should also be tested to determine if the probabilities of induction are conditional or independent.

4.4.4 Cell Systems for Neoplastic Transformation

The mechanisms whereby single or multiple somatic mutations result in neoplastic transformation--i.e., in unregulated growth--should be studied further using cells in culture. However, significant limitations exist in reference to the cell systems that are available for quantitative radiobiological work as noted below.

4.4.4.1 Endpoints of Neoplastic Transformation

Oncogene products that can play a role in growth regulation, including cell differentiation, in principle can do so in a variety of ways (see Sections 2.3 & 2.4, and Fig. 2.4). In addition, altered cell communication has been implicated in neoplastic transformation (Yamasaki and Fitzgerald, 1988). Accordingly, the manifestations of altered oncogene action can be expected to be evident in a variety of cellular changes. For quantitative study, essentially only two endpoints are available at present.

The first is based upon a morphological alteration in the appearance of a colony of transformed cells. This endpoint is used to assess transformation in mouse C3H 10T1/2 cells by "focus formation," a dense colony of cells in a confluent sheet of normal-appearing cells (Reznikoff et al., 1973), and in Syrian hamster embryo cells by the altered organization of the cells in an individual, discrete colony well separated from colonies of normal cells (Borek and Sachs, 1966).

The second method for detecting a transformed phenotype uses a human/human cell hybrid--that is a HeLa cell hybridized with a human skin fibroblast--and is based upon the expression of a new cell-surface protein by a colony of transformed cells, and the ability to detect the appearance of that protein by a staining procedure employing a monoclonal antibody to detect the protein (Redpath et al., 1987).

Cellular-based endpoints are needed to measure quantitatively the radiation-induced changes that can be indicative of neoplastic transformation.

It is not known if the dependence of transformation frequency on the radiobiological parameters that have been measured reflect, in some unique way, the particular cellular assay of transformation that was used. For example, evidence has been presented that interference with cell-to-cell communication underlies the focus formation of 10T1/2 cells in a confluent layer of normal cells (Mordan et al., 1983). Communication impairment is probably not involved in the assay of transformed Syrian hamster embryo cells because the transformed phenotype is identified as a morphologically altered, discrete colony, well-separated from nontransformed cells. In order to determine if radiobiological transformation depends, qualitatively or quantitatively, on the underlying molecular basis for a particular phenotypic change, a number of different endpoints are needed. A number of *in vitro* correlates of neoplastic transformation *in vivo* have been identified in past studies including reduced serum dependence, increased cellular density at confluence, loss of anchorage dependence for growth, aneuploidy, and immortalization. These correlates could be useful starting points for the development of additional quantitative endpoints of transformation.

4.4.4.2 Molecular Characterizations of Transformed Phenotypes

To be able to relate particular cellular endpoints to the somatic mutation(s) that may give rise to them, the molecular basis for the transformed phenotype needs to be identified. As mentioned above, in the 10T1/2 cell system communication is believed to play a role (Mordan et al., 1983), and the activation of, as yet, unidentified genes also has been implicated (Borek et al., 1987). How these particular changes relate to somatic mutation(s) and the phenotypic expression of neoplastic growth has not been worked out.

For each of the endpoints of neoplastic transformation that is used, the molecular mechanism upon which it depends needs to be determined. It is likely, for some endpoints, that several different molecular changes may give rise to the same end effect. A further requirement is to determine if, and how, radiobiological dependencies reflect particular molecular mechanisms.

4.4.4.3 Transformation Endpoints and Oncogene/Anti-oncogene Activity

In addition to knowing what molecular mechanism(s) may underlie a particular endpoint of neoplastic transformation, it is necessary to have systems, which respond to the specific changes in cancer genes, that are associated with human neoplasms. As noted in Section 3.4, progress has been made in identifying these changes, and, in at least one instance, a specific oncogene mutation has been associated with a radiation-induced tumor (Guererro et al., 1984). Cellular assay systems are needed in which transformation endpoints are a consequence of the same kind(s) of somatic mutations that appear in human tumors (Section 3.4) in order that their dependence on radiobiological parameters can be determined.

4.4.4.4 Species of Origin

In addition to tissue of origin, it is necessary that cells should be available for transformation measurements derived from species appropriate to the objectives of the study. Because the radiogenic human tissues are known, human cells that are derived from those tissues would be particularly useful. However, in general rodent cells have proven to be the easiest to cultivate, and thus far two of the three cell systems available for study are of rodent origin. More

human cells in culture are needed for transformation measurements. But also, for a given tissue, cells from rodents, dogs, and monkeys would facilitate the biological extrapolation that is required to relate measurements of tumorigenesis in rodents to tumorigenesis in people (see Section 3.5).

4.5 CHROMOSOME ABERRATIONS

As discussed in Tasks 2 and 3 (Sections 2.3 and 3.4), chromosomal aberrations play an important role in mediating lethal, mutagenic, and oncogenic effects of ionizing radiations. A better understanding of the mechanism of aberration induction is important, therefore, for understanding factors that affect their induction by radiation. This knowledge could be important for the quantitative assessment of risk associated with low-doses and low-dose-rate exposures to ionizing radiation.

4.5.1 Somatic Cells

Although familial dispositions to cancer are known to be transmitted via mutations in germ-line cells, neoplastic growth is expressed by somatic cells. Hence, somatic cells in culture, preferably diploid, lend themselves to studies of aberration production.

4.5.1.1 Metaphase Aberrations, Classical Approach

The classical approach in radiation cytogenetics, which is still largely current, involves the measurement of aberrations in the first mitosis after the cells were irradiated. This process is facilitated by the use of a chemical (e.g., colcemid) to accumulate cells in metaphase. For cells irradiated in the G_1 - or G_0 -phases of the cell cycle, the aberrations observed in this way usually include various asymmetrical chromosome-types, intra- and interchanges (e.g., interstitial deletions, centric rings, and dicentrics with their accompanying acentric fragments). For cells irradiated during the S- or G_2 -phases, the aberrations include terminal deletions, and both symmetrical and asymmetrical exchanges and deletions.

Most asymmetrical, chromosome-type exchange aberrations are lethal. Nonetheless, a measurement of their induction can give a good estimate of the frequency of the more stable symmetrical exchanges which require special techniques to be detected. The latter class of aberrations includes many of the chromosomal changes associated with neoplasia. Symmetrical, chromosome-type exchanges can be measured by detailed analyses of karyotype, but this procedure is not feasible for the low-frequency events that would be induced by protracted low-doses if conventional cytogenic techniques are applied. Methods have become available during the past few years that may resolve this problem (see Section 4.5.1.2).

Data for human cells have been derived largely from lymphocytes stimulated to enter the cell cycle by treatment with a mitogen, such as phytohemagglutinin, following radiation exposure in vivo. More accurate assessments of dose-response relationships have been obtained with whole blood or lymphocyte cultures irradiated in vitro.

Various factors significantly influence quantitative measurements of aberrations. Examples include the variation in radiosensitivity of cells at

different positions in the cell cycle (e.g., G₀, G₁, S, G₂, and M phases) with respect to both differential mitotic delays, and differential sensitivity for aberration induction; the limited lifetime of lymphocytes *in vivo*; and the selective elimination of cells with certain aberrations after the first or subsequent generations following irradiation. Knowledge of the influence of these and other factors is critical for accurate and meaningful assessments of dose-response measurements. Much experimental data have been of little use for one or more of the reasons noted. On the other hand, when these complicating factors had been accounted for, more reliable and useful data were obtained.

An example of a careful study of the dose-effect relationship for the production of dicentrics in human lymphocytes irradiated in the G₀-phase and detected in the first metaphase, is that of Lloyd et al. (1975). Because over 14,000 metaphases were scored in obtaining data for doses of zero, 5, 10, 25, and 50 cGy, this study illustrates the resolution that may be achievable by classical methods. Together with other carefully performed work, these studies established that, for chromosome-type asymmetrical interchanges, a linear-quadratic dose-response relationship applies of the form,

$$I = \alpha D + \beta D^2,$$

where I is the incidence due to dose D, and α and β are constants.

4.5.1.2 Metaphase Aberrations, New Approaches

For measurement of chromosomal aberrations at metaphase, considerable potential for the improvement of cytogenetic assays lies in the use of molecular probes, which can be labelled with a useful "tag," and that bind specifically to parts, or all, of certain chromosomes. Among these techniques are the hybridization *in situ* of nucleic acid probes that bind to certain specific chromosomes, or classes of chromosomes, and can be visualized by fluorescent optical techniques (see Pinkel et al., 1986; 1988). This method of labeling can be used to distinguish human from rodent chromosomes in hybrid cells, and even specific human chromosomes in a normal diploid karyotype. With such probes, it should be possible to identify reciprocal translocations that are relevant to mutational and oncogenic transformation events. If such exchanges are stable, or if their rate of elimination is known, it should also be possible to measure the accumulated effect of low-level radiation exposure over many cell generations.

With respect to changes in the normal chromosomal locations of genes, or other specific DNA sequences that may be present in low copy number, even higher resolution than the foregoing should be possible by the methodology of *in situ* DNA hybridization. Resolution would be least for metaphase chromosomes, higher for prophase chromosomes, and still higher for prematurely condensed interphase chromosomes. If complications associated with, for example, chromatin looping can be resolved, extremely high resolution may be attainable for interphase nuclei.

The use of non-DNA probes of certain chromosome regions is also of interest. For example, attempts have been made to use fluorescently labeled antibodies against kinetochore protein, coupled to a fluorescent DNA stain, in a flow-cytometric assay that enables the measurement of the frequency of chromosomes either without a centromere (an acentric fragment), or with two centromeres (a dicentric chromosome). Other nucleic acid probes, for example

specific for the regions of the centromere or telomere, are also becoming available. These techniques should be compatible with the rapid screening of large numbers of cells for changes induced even at low frequencies.

Further advances in the technologies for the rapid screening of human cells should be sought in microscope-based, image-analysis systems employing banding techniques, or in combination with probes such as those mentioned above (Lundsteen and Piper, 1989). Image analysis could offer considerable promise for high-resolution measurements of dose-effect relationships in the low-dose region.

4.5.1.3 Micronucleus Assay

Aberrations readily seen in the first mitosis after irradiation of G_0 - or G_1 -phase cells generate acentric chromosome fragments, and most of these form what is called a "micronucleus" in one of the daughter cells. Scoring the frequency of such micronuclei, therefore, can provide an index of the aberration frequency, provided proper account is taken of the nature of the process. This method has considerable potential for use in automated scoring systems for large numbers of cells--e.g., flow cytometry or microscope-based image analysis--provided complicating factors can be satisfactorily accounted for.

4.5.1.4 Premature Chromosome Condensation

Johnson and Rao (1970) discovered that factors contained in a cell during mitosis can cause the chromatin of an interphase cell to condense prematurely when the two cells are fused. This technique has been used to visualize damage to interphase chromosomes immediately after irradiation and to study the rejoining (and mis-rejoining) of chromosome breaks after irradiation (see Waldren and Johnson, 1974; Hittelman and Rao, 1974; Cornforth and Bedford, 1983a,b; Pantelias and Maille, 1985). The number of breaks per unit dose observable immediately after irradiation has been found to be an order of magnitude greater than the number of aberrations evident after cells have progressed to mitosis. [In the studies of Cornforth and Bedford (1983a,b), the number were 20-fold greater.] The initial frequency of breaks was linear with dose, over the range of dose from 10 to 600 cGy, and the curve extrapolated to zero breaks at zero dose (Cornforth and Bedford, 1983a,b). However, Cornforth and Bedford found that, by several hours after the irradiation, the dose-response curve for the production of excess PCC fragments could be fitted more appropriately by a linear-quadratic curve and one that was similar to the curve observed for mitotic aberrations.

Hence, what is measured by the method of premature chromosome condensation is likely to reflect more accurately the lesions that are present immediately after exposure and before they rejoin or misrejoin to form the aberrations that may be evident at mitosis. For the detection of an initial form of chromosomal damage, the method is the most sensitive that is available. The primary usefulness of the technique of premature chromosome condensation is likely to center around studies of the processes involved in aberration formation that occur shortly after irradiation. In addition, the method could be quite useful for high-resolution gene mapping (see Section 4.5.1.2).

4.5.2 Germ-Line Cells

Cytological techniques have been used to study translocations induced in spermatogonial cells by the examination of spermatocytes (Preston and Brewen,

1973), and to study aberrations induced in mouse oocytes by examining the cells in metaphase I (see Brewen, et al., 1976). The resolution of the method has not been as high as that achieved with lymphocytes by Lloyd et al. (1975); still, the work with germ cells has served quite well for comparisons of dose-effect relationships, and their dependencies on dose protraction, in reference to mutation studies using specific-locus tests.

Radiation damage reflected by alterations in the synaptonemal complex was studied by Cawood and Breckon (1983). The synaptonemal complex is a protein structure that, among other things, facilitates the pairing, or synapsis, of homologous chromosomes during the pachytene stage of the first meiotic prophase. Using primary spermatocytes from the hamster, Cawood and Breckon compared the incidence of multivalent structures--which were formed by chromosomes carrying aberrations pairing with their normal homologues--in diakinesis/metaphase chromosomal spreads with what they observed in mid-to-late pachytene synaptonemal complexes. For a given dose, they found about a 2-fold increase in the incidence of the latter. Deletions, duplications, translocations, and inversions usually are the causes of the irregularities in synaptonemal complexes. Because by mid-to-late pachytene in spermatocytes adjustments in the synapses may have occurred that could have obscured a proportion of the abnormal configurations, a better stage in meiosis would have been early pachytene. This area of aberration production in germ-line cells should receive additional attention as suggested by Holm et al., (1982).

4.6 EXPERIMENTAL ANIMAL SYSTEMS

Studies of experimental radiation oncogenesis in animals have been used extensively to provide information on both risk estimation and the elucidation of mechanisms of radiation action. In the past, large-scale experiments to measure tumor induction in animals have led to a number of concepts in radiation-induced neoplasia. It is not likely, however, that an understanding of mechanisms will be significantly advanced by additional studies of this type unless they are specifically addressed to questions whose relevance to mechanism justifies the large expense involved. Although progress has been made in the development of animal models as part of systems for research on the cellular and molecular bases of radiation oncogenesis, further developments and new models are needed as indicated below.

4.6.1 Murine Myeloid Leukemia

Radiation-induced acute myeloid leukemia in mice has been associated with a specific defect in chromosome 2 and deregulation of the production of a growth factor, Il-1 (Silver et al., 1987; 1990a,b; see Section 3.3.4.3). The chromosome rearrangement seems to be specific to radiation-induced, and not to spontaneous, leukemias (Haran-Ghera, 1989). A feature that is emerging from these studies is that certain break points exist in the chromosomes of murine cells and, in the instance of chromosome 2, a break point is close to the genes that code for the subunits of Il-1. Following exposure to a variety of clastogenic agents, specific break points have been found to exist in human cells, and a large proportion of these are also found in human neoplasms (Yunis et al., 1987).

Break points can be identified by the banding of chromosomes fixed and treated in mitosis. However, the process of microscopically examining individual spreads of fixed, banded chromosomes is slow, tedious, and requires highly

skilled observers. To advance significantly the examination of chromosomes and the aberrations in them that are associated with different growth states of a particular neoplasm, the techniques of microscopic image analysis should be applied and improved (see Lundsteen and Piper, 1989). These techniques should be capable of levels of resolution, and speed of analysis, not achievable via the use of conventional cytological techniques. In addition, the application of computer-driven data analysis and storage, in all likelihood, could be made an integral part of the pattern recognition equipment.

4.6.2 New Systems for Experimental Radiation Oncogenesis

In addition to the conventional methods of research with cells in culture and inbred animals, during the past decade, technical advances have been made in which cells and animals are used together for oncogenesis research. *In vitro/in vivo* systems in some cases have allowed the examination of mechanisms at stages ranging from initiation to the final development of the neoplasm. The opportunities to examine mechanisms with transgenic animals, particularly mice, should also allow the coupling of molecular/cell studies to studies of tumorigenesis.

4.6.2.1 *In Vitro/In Vivo* Mixed Systems, Mammary and Thyroid Tissues

Both mammary epithelial and thyroid follicular epithelial cells have been adapted into systems which allow quantitation of "clonogenic" (viable, transformable) cells from these specific tissues (Clifton, 1986; Clifton et al., 1986; Ullrich, 1986). Using this approach, plus the appropriate hormones, the clonogens neoplastically transformed by radiation have been quantitated by the development of neoplasms after transplantation back into syngeneic animals. Insights into the relative frequency of radiation initiation of progenitor cells and the importance of hormones in oncogenesis have emerged from such studies (see Sections 1.5.2, 1.5.4, and 3.6.3.3).

In vitro/in vivo mixed systems could be used to develop improved quantitative relationships between cell initiation and tumor induction and the factors that affect this relationship. The work needs to be extended to determine how useful the models will be in the low dose range. Similar studies need to be performed with cells from different organs to determine if tissues radiosensitive, or resistant, to oncogenesis show similar or dissimilar relationships. These models also could be readily applied to studying the effects of host influences, such as hormonal balance and immune responses, on oncogenesis.

4.6.2.2 *In Vitro/in Vivo* Mixed System, Transformation of Tracheal Epithelium

Rat tracheal epithelial cells have been exposed to radiation either *in vitro*, as a cell suspension, or *in situ* (Terzaghi-Howe, 1989). Following the irradiation of cultured cells, they were implanted subcutaneously in syngeneic animals. Phenotypic changes induced by radiation have been compared in cells *in vitro* and *in vivo* in the implanted tracheas. This system has been useful in revealing the importance of growth factors (e.g., TGF-beta) and cell-cell interaction in radiation-induced cell transformation (Terzaghi-Howe, 1989; see Sections 1.5.3 and 3.6.3.2).

Because most radiation-induced neoplasms of the respiratory tract are of the bronchi or deep lung, there is need to develop a model similar to the

tracheal system with epithelial cells that are the more likely targets for human respiratory oncogenesis. This may require a larger animal model than the rat, such as the dog or non-human primate. It should be determined whether the same mechanisms exist for tracheal and bronchial or bronchi-alveolar cells.

4.6.2.3 Transgenic Mice

Mice carrying stable oncogenes, which have been selectively inserted into the genomes of their cells, are being used to study *in vivo* effects of oncogenesis (Hanahan, 1988, 1989; Pattengale *et al.*, 1989). Specific oncogenes can be shown to have different effects on different cells and tissues, and such studies have been useful in demonstrating the multistep nature of oncogenesis (see Section 3.6.3.4).

The effects of radiation on oncogenesis in transgenic mice could be an effective way to elucidate how radiation interacts with and/or specifically activates particular oncogenes. Oncogenes, whose role in particular neoplasms has been implicated from other studies, could be tested to see if they do respond to radiation induction. Further, by appropriate timing, the ability of radiation to initiate, promote, or cause a neoplasm to progress could be tested and if, and how, these actions are affected by protraction of the exposure.

4.7 SUMMARY

Although many technical innovations for the study of cellular and molecular biology have been introduced in the past 10 to 15 years, effectively to mount a research program in radiation oncogenesis further developments are needed. These include improvements in:

- 1) The measurement of the various types of lesions in genomic DNA particularly in reference to their production, repair, and misrepair;
- 2) The techniques for the cultivation of human and animal cells *in vitro* so that the properties of the types of cells of interest can be quantitatively studied in reference to mutation and neoplastic transformation;
- 3) The improvement and application of rapid methods of image analysis for the study of chromosome aberrations; and
- 4) The expanded use of cell-animal systems, including the use of transgenic animals, so that the experimental facility of cells in culture can be effectively coupled to the *in vivo* influences that are relevant to tumorigenesis.

Efforts to improve the materials and methods that are currently available also should include the need to measure changes induced by low doses, and after protracted exposures as well. Although studies of the mechanism of radiation oncogenesis do not have to be exclusively pursued in the context of "weak signals," critical issues, like the presence or absence of thresholds, may have to be examined in the dose region of primary interest. Hence, the techniques for the measurement of molecular and cellular changes should be improved to permit the sensitivity and resolution that would be required.

In the last analysis, radiation-induced neoplasms can only be demonstrated *in vivo*. For this reason, tumor formation in experimental animal systems has to be anticipated as an essential endpoint in a radiation research program. It is not recommended, however, that large-scale experiments, simply to measure the dose dependence of tumorigenesis, should be undertaken. Animal measurements, including the use of xenographs to measure neoplastic properties induced in human cells, should be undertaken only when they are designed to address relevant cellular/molecular questions.

4.8 REFERENCES

- Ager, D. D., W. Dewey, K. Gardiner, W. Harvey, R. T. Johnson, and C. A. Waldren, "Measurement by Pulsed Field Gel Electrophoresis (PFGE) of Radiation-Induced Double Strand Breaks." Radiation Research, (in press), 1990.
- Barlow, D. and H. Lehrach, "Genetics By Gel Electrophoresis: The Impact of Pulsed Field Gel Electrophoresis on Mammalian Genetics." Trends in Genetics 3, 167-171, 1987.
- BEIR V Report, "Health Effects of Exposure to Low Levels of Ionizing Radiation." National Research Council, Washington, D.C., 1990.
- Blocher, D., M., Einspenner, and J. Zajackowski, "CHEF Electrophoresis, a Sensitive Technique for the Determination of DNA Double-Strand Breaks." International Journal of Radiation Biology 56, 437-448, 1989.
- Borek, C., A. Ong, and H. Mason, "Distinctive Transforming Genes in X-Ray-Transformed Mammalian Cells." Proceedings of the National Academy of Sciences USA 84, 794-798, 1987.
- Borek, C. and L. Sachs, "In Vitro Cell Transformation by X-Irradiation." Nature 210, 276-278, 1966.
- Bradley, W. E. C., "Conditional Instability of Induced Variants of CHO: Transient Sensitivity to Contact with Wild Type Cells." Experimental Cell Research 129, 251-261, 1980.
- Bradley, W. E. C., and F. Laviolette, "Low Persistence of the Induced Mutant Phenotype in Chinese Hamster Cells." Mutation Research 210, 303-311, 1989.
- Bredberg, A., K. Kraemer, and R. M. Seidman, "Restricted Ultraviolet Light Mutational Spectrum in A Shuttle Vector Propagated in Xeroderma Pigmentosum Cells," Proceedings of the National Academy of Sciences USA 83, 8237-8277, 1986.
- Breimer, L.H., "Ionizing Radiation-Induced Mutagenesis." British Journal of Cancer 57, 6-18, 1988.
- Brewen, J.G., H. S. Payne, and R. J. Preston, "X-ray Induced Chromosome Aberrations in Mouse Dictyate Oocytes. I. Time and Dose Relationship." Mutation Research 35, 111-120, 1976.
- Bridges, B., B. Butterworth, B. and B. Weinstein, B, eds., Banbury Report Number 13: Indicators of Genotoxic Exposure, Cold Spring Harbor Laboratories, New York, 198, 1982.
- Calos, M. P., "Mutation of Autonomously Replicating Plasmids." In: Gene Transfer, Ed, R. Kucherlapati, Plenum Press, New York, pp. 243-262, 1986.
- Cawood, A. H. and G. Breckon, "Synaptonemal complexes as indicators of induced structural change in chromosomes after irradiation of spermatogonia." Mutation Research 122, 149-145, 1983.

- Clifton, K.H., "Thyroid Cancer: Reevaluation of an Experimental Model for Radiogenic Endocrine Carcinogenesis." In: Radiation Carcinogenesis, eds., A.C. Upton, R.E. Albert, F.J. Burns, and R.E. Shore, Elsevier, New York, pp. 181-198, 1986.
- Clifton, K.H., M. A. Tanner, and M. N. Gould, "Assessment of Radiogenic Cancer Initiation Frequency per Clonogenic Rat Mammary Cell *in Vivo*." Cancer Research 46, 2390-2395, 1986.
- Contopoulou, C.R., V. E. Cook, and R. K. Mortimer, "Analysis of DNA Double Strand Breakage and Repair Using Orthogonal Field Alternation Gel Electrophoresis." Yeast 3, 71-76, 1987.
- Cornforth, M. N. and J. S. Bedford, "High Resolution Measurement of Breaks in Prematurely Condensed Chromosomes by Differential Staining." Chromosoma 88, 315-318, 1983a.
- Cornforth, M. N. and J. S. Bedford, "X-ray Induced Breakage and Rejoining of Human Interphase Chromosomes." Science 222, 1141-1143, 1983b.
- Das, G., J. W. Stewart, and F. Sherman, "Mutational Alterations Induced in Yeast by Ionizing Radiation." Mutation Research 163, 233-438, 1986.
- Elkind, M. M., C. K. Hill, and A. Han, "Repair and Misrepair in Radiation-Induced Neoplastic Transformation." In: Carcinogenesis, eds., E. Huberman and S. H. Barr, Raven Press, New York, pp 317-336, 1985.
- Elkind, M. M., "The Initial Part of the Survival Curve: Implications for Low-Dose, Low-Dose-Rate Responses." Radiation Research 71, 9-23, 1977.
- Evans, H. H., J. Mencl, M-F Horng, M. Ricanti, C. Sanchez, and J. Hozier, "Locus Specificity in the Mutability of L5178Y Mouse Lymphoma Cells: The Role of Multilocus Lesions." Proceedings of the National Academy of Sciences USA 83, 4379-4383, 1986
- Gardiner, K., P. Watkins, M. Munke, H. Drabkin, C. Jones, and D. Patterson, "Partial Physical Map of Human Chromosome 21." Somatic Cell and Molecular Genetics 14, 623-638, 1988.
- Gorgojo, L., and J. B. Little, "Expression of Lethal Mutants in Progeny of Irradiated Mammalian Cells." International Journal of Radiation Biology 55, 619-630, 1989.
- Grosovsky, A., J. de Boer, P. de Jong, E. Drobetsky, and B. Glickman, "Base Substitutions, Frameshifts, and Small Deletions Constitute Ionizing Radiation-Induced Point Mutations in Mammalian Cells." Proceedings of the National Academy of Sciences, USA 85, 185-188, 1988.
- Guererro, I., A. Villasante, A. Corces, and A. Pellicer, "Activation of a c-K-ras Oncogene by Somatic Mutation in Mouse Lymphomas Induced by Gamma Radiation." Science 255, 1159-1162, 1984.
- Hanahan, D., "Dissecting Multistep Tumorigenesis in Transgenic Mice." Annual Review of Genetics 22, 479-519, 1988.

- Hanahan, D., "Transgenic Mice as Probes into Complex Systems." Science 246, 1265-1274, 1989.
- Haran-Ghera, N., "Radiation-Induced Deletion of Chromosome 2 in Myeloid Leukemogenesis." Current Topics in Microbiology and Immunology 149, 35-41, 1989.
- Hittleman, W. N. and P. N. Rao, "Premature Chromosome Condensation. I. Visualization of X-ray Induced Chromosome Damage in Interphase Cells." Mutation Research 23, 251-258, 1974.
- Holm, P. B., S. W. Rasmussen, and D. von Wettstein, "Ultrastructural Characterization of the Meiotic Prophase: A Tool in the Assessment of Radiation Damage in Man." Mutation Research 95, 45-59, 1982.
- Hutchinson, F., "Use of Data From Bacteria to Interpret Data on DNA Damage Processing in Mammalian Cells." Mutation Research 220, 269-278, 1989.
- Johnson, R. T. and P. N. Rao, "Mammalian Cell Fusion. II. Induction of Premature Chromosome Condensation in Interphase Nuclei." Nature 226, 717-722, 1970.
- Kucherlapati, R., "Mutation of Autonomously Replicating Plasmids." In, Gene Transfer, Plenum Press, New York, pp 243-262, 1986.
- Lai, E., B. W. Birren, S. M. Clark, M. I. Simon, and L. Hood, "Pulsed Field Gel Electrophoresis." BioTechniques 7, 34-41, 1989.
- Liber, H. L., P.-M. Leong, V. H. Terry, and J. B. Little, "X-rays Mutate Human Lymphoblast Cells at Genetic Loci That Should Only Respond to Point Mutations." Mutation Research 163, 91-98, 1986.
- Liber, H. L., K. M. Call, and J. B. Little, "Molecular and Biochemical Analyses of Spontaneous and X-ray-Induced Mutants in Human Lymphoblast Cells," Mutation Research 178, 143-153, 1987.
- Lloyd, D. C., R. J. Purrott, G. W. Dolphin, D. Bolton, A. A. Edwards, and M. J. Corp, "The Relationship Between Chromosome Aberrations and Low LET Radiation Dose to Human Lymphocytes." International Journal of Radiation Biology 28, 75-83, 1975.
- Lundsteen, C. and J. Piper (eds.), Automation of Cytogenetics, Springer-Verlag, Berlin, 316 pages, 1989.
- Maxam, A. M. and W. Gilbert, "A New Method For Sequencing DNA." Proceedings of the National Academy of Sciences USA 74, 560-564, 1977.
- Mordan, L. J., J. E. Martner, and J. S. Bertram, "Quantitative Neoplastic Transformation of C3H/10T1/2 Fibroblasts." Cancer Research 43, 4062-4067, 1983.
- Mothersill, C., and C. Seymour, "The Influence of Lethal Mutations on the Quantification of Radiation Transformation Frequencies." International Journal of Radiation Biology 51, 723-729, 1987.
- Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich, "Specific Enzymatic Amplification of DNA *in Vitro*: The Polymerase Chain Reaction."

The Cold Spring Harbor Symposium on Quantitative Biology 51, 263-273, 1986.

- Pantelias, G.E. and H. D. Maille, "Direct Analysis of Radiation-Induced Chromosome Fragments and Rings in Unstimulated Human Peripheral Blood Lymphocytes by Means of the Premature Chromosome Condensation Technique." Mutation Research 149, 67-72, 1985.
- Pattengale, P. K., T. A. Stewart, A. Leder, E. Sinn, W. Muller, I. Tepler, E. Schmidt, and P. Leder, "Pathology and Molecular Biology of Spontaneous Neoplasms Occurring in Transgenic Mice Carrying and Expressing Activated Cellular Oncogenes." American Journal of Pathology 135, 39-61, 1989.
- Pinkel, D., J. Landegent, C. Collins, J. Fuscoe, R. Segraves, J. Lucas, and J. Gray, "Fluorescence In Situ Hybridization with Human Chromosome Specific Libraries: Detection of Trisomy 21 and Translocations of Chromosome 4." Proceedings of the National Academy of Sciences, USA 86, 9138-9142, 1988.
- Pinkel, D., T. Straum, and J. W. Gray, "Cytogenetic Analysis Using Quantitative, High-Sensitivity, Fluorescence Hybridization." Proceedings of the National Academy of Sciences, USA 83, 2934-2938, 1986.
- Preston, R. J. and J. G. Brewen, "X-ray Induced Translocations in Spermatogonia. I. Dose and Fractionation Responses In Mice." Mutation Research 19, 215-223, 1973.
- Randerath, K., M. Reddy, and R. Gupta, " ³² P-labeling Test for DNA Damage." Proceedings of the National Academy of Sciences, USA 78, 6126-6129, 1981.
- Redpath, J. L., C. Sun, M. Colman, and E. J. Stanbridge, "Neoplastic Transformation of Human Cells by Gamma-Rays: A Quantitative Assay." Radiation Research 110, 468-472, 1987.
- Reznikoff, C.A., D. W. Brankow, and C. Heidelberger, "Establishment and Characterization of a Cloned Line of C3H Embryo Cells Sensitive to Post Confluence Inhibition of Division." Cancer Research 33, 3231-3238, 1973.
- Roza, L., K. Van der Wulp, S. MacFarlane, P. Lohman, and R. Baan, "Detection of Cyclobutane Thymine Dimers in DNA of Human Cells With Monoclonal Antibodies Raised Against a Thymine Dimer Containing Tetranucleotide." Photobiology 48, 627-634, 1986.
- Sanger, F., S. Nicklen, and A. Coulson, "DNA Sequencing With Chain-Terminating Inhibitors," Proceedings of the National Academy of Sciences, USA 74, 5463-5466, 1977.
- Silver, A. R. J., G. Breckon, W.K. Masson, D. Malowany, and R. Cox, "Studies on Radiation Myeloid Leukemogenesis in the Mouse." In: Radiation Research, Proceedings of the 8th International Congress of Radiation Research, eds., E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scott, Taylor and Francis, London, 2, 494-500, 1987.
- Silver, A. R. J., G. Breckon, W. K. Masson, J. Adam, J. Boulwood, and R. Cox, "Radiation-Induced Chromosome 2 Rearrangement and Initiation of Murine Acute Myeloid Leukemia." Radiation Research (In Press) 1990a.

- Silver, A. R. J., G. Breckon, W. K. Masson, J. Adam, A. R. Shaw, and R. Cox, "Interleukin-1 β Gene Deregulation Associated with Chromosomal Rearrangement: A Candidate Initiating Event for Murine Radiation-Myeloid Leukemogenesis?" Molecular Carcinogenesis (in press), 1990b.
- Stamato, T. D., R. Weinstein, B. Peters, J. Hu, B. Doherty and A. Giaccia, "Delayed Mutation in Chinese Hamster Cells." Somatic Cell Molecular Genetics 13, 57-66, 1987.
- Stamato, T. D., and N. Denko, "Asymmetric Field Inversion Gel Electrophoresis: A New Method for Detecting Double-Strand Breaks in Mammalian Cells," Radiation Research 121, 196-205, 1990.
- Terzaghi-Howe, M., "Induction of Preneoplastic Alterations by X-Rays and Neutrons in Exposed Rat Tracheas and Isolated Tracheal Epithelial Cells." Radiation Research 120, 352-363, 1989.
- Thacker, J., "The Use of Recombinant DNA Techniques To Study Radiation-Induced Damage, Repair and Genetic Change In Mammalian Cells." International Journal of Radiation Biology 50, 1-30, 1986
- Thacker, J., M. A. Stephens, and A. Stretch, "Mutation to ouabain-resistance in Chinese Hamster Cells: Induction by Ethylmethanesulphonate and Lack of Induction by Ionizing Radiation." Mutation Research, 51, 255-263, 1978.
- Ulanovsky, L., G. Drouin, and W. Gilbert, "DNA Trapping Electrophoresis." Nature 343, 190-192, 1990.
- Ullrich, R. L., "The Rate of Progression of Radiation-Transformed Mammary Epithelial Cells after Low-Dose-Rate Neutron Irradiation." Radiation Research 105, 68-75, 1986.
- Urlaub, G., P. J. Mitchell, E. Kas, L. Chasin, V. Furnanage, P. Myoda, J. Hamlin, "Effect of Gamma Rays at the Dihydrofolate Reductase Locus: Deletions and Inversions." Somatic Cell and Molecular Genetics 12, 555-566, 1986.
- Waldren, C. A. and R. T. Johnson, "Analysis of Interphase Chromosome Damage by Means of Premature Chromosome Condensation After X-ray and Ultraviolet Irradiation." Proceedings of the National Academy of Sciences, USA 71, 1137-1141, 1974.
- Yamasaki, H., and D. J. Fitzgerald, "The Role of Selective Junctional Communication and Tumor Promoter-Inhibited Communication in Cell Transformation." In: Tumor Promoters, eds, R. Langenbach, C. J. Barrett, and J. Elmore, Raven Press, New York, pp. 131-147, 1988.
- Yandell, D.W., T. P. Drya, and J. B. Little, "Somatic Mutations at a Heterozygous Autosomal Locus in Human Cells Occur More Frequently by Allelic Loss Than by Intragenic Structural Alterations." Somatic Cell Molecular Genetics 12, 255-263, 1986.
- Yunis, J. J., A. L. Soreng, and A. E. Bowe, "Fragile Sites Are Targets of Diverse Mutagens and Carcinogens." Oncogene 1, 59-69, 1987.

Task 5

"Facilities for a Radiation Research Program"

5.1 INTRODUCTION

To mount effectively a research program in radiation biology directed at the mechanisms of radiation-induced oncogenesis, particularly in reference to protracted low doses of low-LET radiation, a range of facilities are required. Not only are the laboratories and equipment required typical of what has been available since the 1950's, but also the facilities and instruments that have emerged during the past 15 years as concomitants of new developments in cell and molecular biology. Principal among the latter have been those devices that enable the determination of changes produced in the genetic code of a cell.

In this Task, the major types of experimental procedures in a program of research are noted and categorized, the kinds of laboratories in which these procedures could be undertaken are described, and operating units at existing institutions are identified that are generally recognized to have facilities as well as the personnel effectively to pursue one or more parts of an integrated program. In the latter connection, it is important to recognize that the list of laboratories is not intended to be exhaustive, nor to suggest that others do not exist capable of effectively participating in the research program.

5.2 CATEGORIES OF EXPERIMENTAL PROCEDURES

5.2.1 Molecular Techniques

The principal experimental departure of this program rests on the ability to analyze, at the level of the genetic code, the changes principally in somatic cells that are responsible for the loss of cellular growth control. Hence, laboratories, or laboratory units, would be required that have the professional and technical staff to apply the techniques that already have been developed and to introduce new techniques as may be required. The experimental procedures that are available should include: Southern, northern, and western blotting; one- and two-dimensional gel electrophoresis; DNA, RNA, and protein sequencing and methods of synthesizing these molecules; cytogenetic/molecular techniques for locating specific genetic sequences in chromosomes; and methods for identifying cytogenetically, or by other means, chromosomal changes. Items of specialized equipment that would be required for such studies should include: the various devices for gel electrophoresis; instruments and techniques for visualizing and reading the signals, both optical and radioisotopic, generated by the use of electrophoretic separations; equipment for synthesizing DNA, RNA, and proteins; the microscopic instruments for cytogenetic measurements; instruments for the separation and identification of various molecular, subcellular, and viral species; and the computer facilities for storing/analyzing molecular data.

5.2.2 Cellular Techniques

Since its introduction in the late 1940's, the techniques for the cultivation of mammalian cells *in vitro* have been widely applied in biological research. To support a program of research, the facilities and equipment would be needed to cultivate animal and human cells under controlled conditions for the measurement of: cellular endpoints, like cell growth, colony-forming ability, mutation, and transformation; to furnish the hosts for viral studies; and to

Table 5.1. Laboratories Capable of Mounting a Program of Research

Institutional and/or Organizational Unit	Molecular Techniques Available	Cellular Techniques Available	Radiation Facilities ¹	Animal Facilities ²
Argonne National Laboratory, Division of Biological, Medical & Environmental Research	X	X	L & LD' H & HD'	R, D
Brookhaven National Laboratory, Biology and Medical Departments	X	X	L & LD"? H? & HD"?	R, D?
Case Western Reserve University, Department of Radiology	X	X	L & LD"? H? & HD"?	R, D?
Colorado State University, Department of Radiology and Radiation Biology	X	X	L & LD'	R, D
Columbia University Medical School, Radiological Laboratory	X	X	L & LD"? H & HD"?	R, D?
Emory University, Department of Biochemistry	X	X	L & LD"?	R?, D?
Georgetown University Medical School, Department of Radiation Medicine	X	X	L & LD"?	R, D?
Harvard University, School of Public Health	X	X	L & LD' H & HD"?	R, D?
Johns Hopkins University Medical School, Dept. of Radiation Oncology	X	X	L & LD"? H? & HD"?	R, D?
Lawrence Livermore Laboratory, Biomedical Sciences Division	X	X	L & LD"? H? & HD"?	R?, D?
Los Alamos National Laboratory, Life Sciences Division	X	X	L & LD"? H? & HD"?	R, D?
Lovelace Biomedical and Environmental Research Institute	X	X	L & LD"?	R, D?

¹ L: Low-LET, high-dose-rate source
H: High-LET, high-dose-rate source
LD': Low-dose-rate, low-LET source
HD': Low-dose-rate, high-LET source
?: Characteristics of source not known

² R: Rodents
D: Dogs
?: Kind of facilities not known

Table 5.1. Laboratories Capable of Mounting a Program of Research (Cont.)

Institutional and/or Organizational Unit	Molecular Techniques Available	Cellular Techniques Available	Radiation Facilities ¹	Animal Facilities ²
Medical Research Council, Radiobiology Unit, United Kingdom	X	X	L & LD' H & HD'	R, D?
Michigan State University, Carcinogenesis Laboratory and the Department of Pediatrics and Human Genetics	X	X	L & LD''?	R, D?
National Cancer Institute, Radiation Oncology Branch	X	X	L & LD''?	R, D?
National Institute of Radiological Health, Japan	X	X	L & LD''? H & HD''?	R, D?
New York University, Institute of Environmental Medicine	X	X	L & LD''?	R, D?
Oak Ridge National Laboratory, Biology Division	X	X	L & LD' H & HD'	R, D?
Ontario Cancer Institute, Medical Physics Division, Canada	X	X	L & LD'	R, D?
Pacific Northwest Laboratory, Biology Division	X	X	L & LD''? H? & HD''?	R, D
Rochester University, Departments of Biophysics and Microbiology	X	X	L & LD''?	R, D?
Stanford University Medical School, Departments of Biophysics and Radiation Oncology	X	X	L & LD''?	R, D?
University of Arizona Medical School, Department of Radiation Oncology	X	X	L & LD''?	R, D?
University of California at Irvine, Department of Radiological Science	X	X	L & LD''? H? & HD''?	R, D?

¹ L: Low-LET, high-dose-rate source
H: High-LET, high-dose-rate source
LD': Low-dose-rate, low-LET source
HD': Low-dose-rate, high-LET source
?: Characteristics of source not known

² R: Rodents
D: Dogs
?: Kind of facilities not known

Table 5.1. Laboratories Capable of Mounting a Program of Research (Cont.)

Institutional and/or Organizational Unit	Molecular Techniques Available	Cellular Techniques Available	Radiation Facilities ¹	Animal Facilities ²
University of California, Lawrence Berkeley Laboratory	X	X	L & LD'? H & HD'	R, D?
University of California at Los Angeles, Laboratory of Biomedical and Environmental Science	X	X	L & LD'? H & HD'	R, D?
University of California at Riverside, Program in Environmental Sciences	X	X	L & LD'?	R?, D?
University of California at San Diego, Department of Radiology	X	X	L & LD'?	R?, D?
University of California at San Francisco, Lab of Radiobiology and Environmental Research & Dept. of Radiation Oncology	X	X	L & LD'? H? & HD'?	R, D?
University of Chicago, Division of Biological Sciences	X	X	L & LD'? H & HD'?	R, D?
University of Iowa Medical School, Radiation Research Laboratory	X	X	L & LD'?	R?, D?
University of Texas Medical School, M.D. Anderson Hospital and Tumor Clinic, Dept. of Experimental Radiation Therapy	X	X	L & LD'? H & HD'?	R, D?
Wistar Institute, Philadelphia	X	X	L & LD'?	R, D?
Yale University, Department of Therapeutic Radiology	X	X	L & LD'?	R, D?

¹ L: Low-LET, high-dose-rate source
H: High-LET, high-dose-rate source
LD': Low-dose-rate, low-LET source
HD': Low-dose-rate, high-LET source
?: Characteristics of source not known

² R: Rodents
D: Dogs
?: Kind of facilities not known

support the molecular and genetic studies connected with the growth control of cells. Because the application of DNA recombinant techniques frequently requires the use of bacterial or yeast cells, facilities appropriate for the controlled growth of such cells would also be required.

The instruments appropriate for cellular measurements should include: conventional incubators, warm rooms, and cold rooms; light and electron microscopic equipment and ancillary devices for pattern recognition; and instruments for flow cytofluorography and cell sorting. In addition, the more conventional devices for analytical studies in cell biology should be available including computers for the storage and analysis of data.

5.2.3 Irradiation Facilities

Although low-LET radiations would be of primary interest, the overall program of research should seek to have available some sources of high-LET radiation. Hence, in addition to X-rays and various isotopic sources of sparsely ionizing radiation, charged atomic nuclei, like alpha-particles, and fission-spectrum neutrons should be available. The latter radiation would be of interest in a program of research because cellular and animal data already exist connected with such exposures, and because of the industrial and military applications of reactor-generated neutrons.

The program of research also has as a principal objective the dependence of molecular/cellular/tissue endpoints on the protraction of radiation exposures. Hence, both low- and high-LET radiation facilities are needed that would permit not only controlled exposures to graded low doses, but also to doses that are delivered over extended intervals. In the latter context, an extended interval would mean an exposure period that permits an extrapolation to the situation that applies, in the limit, to the public at large. Consequently, some of the sources of radiation in the program should be able to expose the material of interest under controlled and optimal biological conditions, and for periods of hours to months depending on the context and circumstances of the experiment.

5.2.4 Animal Facilities

In some of the laboratories, or laboratory units, it would be necessary to have adequate animal facilities for rodents, at least, and also for dogs and/or monkeys. Animals would be needed for three general purposes. First, they can serve as an analytical tool in the support of cellular/molecular studies. For example, from animals large amounts of cellular material could be obtained, as required, and/or the biological properties of specific molecular or cellular species could be evaluated. Second, animals can be used to test hypotheses related to neoplasia under conditions that can simulate those that apply to people. For example, the genetic changes associated with the metastatic development of a tumor could be examined, or the oncogenic expression of a particular oncogene in a transgenic context. And third, under well-defined circumstances, animals could be used to test hypotheses of how radiation induces tumors, and the factors that are relevant to an extrapolation from animal to human cancer.

5.3 LABORATORIES AND LABORATORY UNITS

Listed in Table 5.1 are the laboratories that are known to have at least the nucleus of the professional staff and effective capabilities to mount a

program of research in at least three of the four areas that have been identified; that is, in at least *molecular techniques*, *cellular techniques*, *radiation facilities*, and *animal facilities*. The underlying premise is that at least the first three areas must be available in order to sustain an integrated program of research. It is also noted that, while a full complement of staff is not essential from the outset, a core of scientists and assistants is needed who have the requisite molecular/cellular experience plus a background in radiobiology. It is assumed that, as required, additions can be made to the core.

Task 6

"Agenda for a Radiation Research Program" *Topics, Methods, and Facilities*

6.1 INTRODUCTION

Research is frequently the most productive when investigators have the freedom to express and to implement their own ideas. Still, to pursue a set program objectives, it is useful if not necessary to have the broad aims of the work clearly defined. In this Task, an outline is developed of the main objectives of a program of research directed at understanding the mechanism(s) of radiation-induced cancer. Although it cannot be guaranteed, it is likely that reductions in uncertainties in estimates of risk would be an outcome of such an understanding.

6.2 RESEARCH TOPICS

In Task 3, Sections 3.2 through 3.6, specific gaps in the understanding of a number issues were identified. The latter Sections should be referred to for more detailed statements of specific research questions. The major topics to which these questions relate are the following:

6.2.1 Oncogenic Characterizations of Neoplastic Growth States

One of the principal outcomes of the discovery of oncogenes, and the application of the methods of DNA recombinant technology that led to them, has been the progress that has been made in the characterization of several human neoplasms. Changes in the properties of certain oncogenes as well as in their regulation have been identified. Chromosomal rearrangements connected with specific neoplasms have also been discovered. The *growth-state diagrams* for some human cancers that have resulted from this work (see Section 3.4) needs to be expanded to all of the human tumors that are known to be radiogenic. Similarly, for reasons explained below, growth state diagrams are required for the corresponding animal tumors and particularly for those tumors for which a body of radiation data already exists.

6.2.2 Radiation-Induced Oncogenic Changes and Their Dependence on Radiological Parameters

It is probable that the growth states of human neoplasms that will be determined will apply to so-called spontaneous tumors. In contrast, experimentally induced animal tumors in the main will be the result of relatively high doses of radiation delivered at high dose rates. The possible dependence upon dose, dose rate, and LET has to be taken into account in order to relate growth-state descriptions of animal tumors to those of human tumors. If growth-state data for radiation-induced human tumors should become available--for example, lung cancer due to exposure to radon--it will be relevant to determine whether or not those neoplasms are characterized by the same or different oncogenic/cellular changes as are spontaneous tumors. In broad terms, therefore, the roles of repair/misrepair processes on the frequencies and types of genetic lesions in radiation oncogenesis should be determined because of the likelihood that such processes will increase in relative importance as the dose is decreased and the exposure time lengthened.

6.2.3 Growth State Transitions

The oncogenic changes that describe a given growth state do not necessarily indicate how the transitions between growth states occur. Not only do these changes have to be consistent with cell viability, but also they must lead at least to progressive advantages in growth in order for a tumor to form. Associated with the mechanism of transitions are questions of *cause and effect*, *independent* and *conditional* or *derivative* changes, and *necessary* and *sufficiency* conditions (see Section 3.4). Because the histo-pathology of radiation-induced neoplasms is not known to differ from those that appear spontaneously, from the mechanism of radiation-induced transitions it should be possible to unravel the transitions between growth states in spontaneous human cancer.

6.2.4 The Interaction of Radiation and Other Environmental Agents

Protracted low doses of ionizing radiation represent only one of a variety of environmental, biologically active agents to which people are more or less continuously exposed. Many of these agents are "genotoxic" and "clastogenic" in that they are known to produce lesions in DNA and/or chromosomal changes, respectively. Some agents may interact with radiation damage in a promotional sense in that by themselves they have no known deleterious effect but, in conjunction with radiation exposure, they can amplify the neoplastic transformation of cells or the yield of tumors. Similarly, there are chemicals that have been found to reduce the radiation yields of genetic changes. Hence, as part of this program of research, an examination should be made of possible interactive effects between radiation and other physical and chemical environmental agents.

6.2.5 Extrapolation from Animal Data to People

Although radiation is well established as a human oncogenic agent, the bulk of the information on the dependence of tumorigenesis on radiation exposure comes from animal studies. In a selective way, no doubt additional data on experimental oncogenesis would be developed in the course of this program. To relate these data to people--both in respect to mechanism of induction plus dependencies on radiobiological parameters--it will be necessary further to develop and to validate principles and methods of extrapolation.

6.3 TECHNIQUES AND FACILITIES

Although the springboard of a research program in cancer induction is the progress that has been made in oncogene research and the methods of DNA recombinant technology that were involved, additional developments in methods, materials, and instruments are needed.

6.3.1 Development of Cell and Animal Systems

The technological accomplishments of the past four decades that enabled the use of animal and human cells for experimentation *in vitro* are recognized as a technical development that led to important advances in a number areas of biological research. Still, in reference to research in oncogenesis, further technical developments are needed. Principal among these are: the need for additional cell lines and endpoints for the measurement of neoplastic transformation (see Sections 3.2 & 4.4); the need for media and growth

conditions that define the conditions of cultivation in absolute terms and that simulate more effectively growth conditions *in vivo* (see Section 3.2 & 4.4); and cell-animal mixed systems in order to exploit the manipulative advantages of cultured cells plus the ability to observe endpoints *in vivo* (see Sections 3.6 & 4.6).

6.3.2 Instrumentation for Improved Laboratory Procedures and Rapid Screening

Because the changes that give rise to transformed phenotypes are events of low frequency, particularly connected with protracted exposures to low doses, methods to enable the detection of "weak" signals are needed. In addition, optimally these methods should have rapid rates of screening because biological material is often labile, and because large numbers of events may have to be analyzed. These requirements indicate three areas of technology that may be usefully applied in a program of research.

Flow Cytometry This technology has already been used successfully in reference to detecting changes in the blood cells of people who may have been exposed to genotoxic agents (see Section 1.7). Similar light-based systems for the detection of transformation-related changes in animal and human cells potentially could be uniquely useful. The method should also permit the selection of those cells for further study that have these phenotypic changes.

Pattern Recognition The detection of chromosomal rearrangements is one area where computer-assisted, microscope-based, pattern-recognition systems have already enabled important findings (see Section 4.5). In addition to further exploitation along these lines, other subtle, low-frequency cellular changes should be detectable by similar methods. As in the instance of flow cytometry, high-speed analysis is critical.

DNA Sequence Analysis The identification of changes in, or the losses from, genes and oncogenes that are connected with neoplasia will be an important part of characterizations of animal and human transformed cells and neoplasms. Improvements in the rate at which such determinations can be made are needed. It is anticipated that developments along such lines may be an outgrowth of the project to sequence the human genome. In addition to the potential usefulness of such information as a base of information against which to identify neoplastic changes, improved methods of sequencing would have direct applicability to a program of radiation research.

6.4 RESEARCH OUTCOMES

The principal deliverable of a program of research on radiation-induced cancer can be expected to be a greater understanding of the processes essential to the growth and development of cells, tissues, and organisms. As noted, the point of departure of this research program would be the advances in the role(s) of oncogenes in controlling normal and aberrant growth, advances that have been derived only to a limited extent from contributions made by radiobiologists. However, the concepts derived from past radiobiological research, and the unique properties of radiation as an inducing agent, suggest that significant contributions to future progress in the knowledge about human neoplasia would come from a program of radiation research.

Uncertainties in the risk of cancer associated with radiation exposure, in addition to background exposure, imply a "cost" to society that is greater than the risk itself. Such costs are reflected in the permissible levels of exposure for the public at large. In the medical, scientific, industrial, and military uses of radiation, the costs are intended to be consistent with the benefits derived therefrom. An outcome of a program of research can be expected to be a narrowing of uncertainties, as the degree of risk is more precisely defined; a lowering of ratios of risk to benefit; and, therefore, a reduction of the costs to society.

A further outcome of an understanding of the mechanisms of radiation-induced cancer would be the likelihood that the frequency of second primary tumors, induced during the course of the treatment of cancer with radiation, would be reduced. As cancer patients have their lives extended by improvements in radiotherapy, the probability increases that they can express a second tumor induced by the therapy itself. A greater understanding of the parameters that influence radiation tumorigenesis should allow therapists to reduce risks while optimizing the benefits of treatment.

6.5 PERSPECTIVE

Radiation, as a factor relevant to health, has assumed a position of public awareness since the use of nuclear weapons in 1945. Technological developments in industry, medicine, and research, both biological and physical, have developed for radiation an indispensable role in contemporary life. It is important to society that the negative, cancer-inducing properties of radiation exposure should be better understood because the clock cannot be turned back.

6.6 SUMMARY

In Task 6, the broad and principal objectives of an agenda for a radiation research program are identified. These were derived from the progress that has been made in describing the oncogenic basis of human cancer, and the innovations in DNA technology that gave rise to them. The need for further information to characterize, in terms of oncogenes, the growth states of human neoplasms is discussed and particularly an understanding of how radiation could cause transitions between successive growth states. The relevance of other biologically active environmental agents to radiation effects is discussed, and the need for a rational basis for extrapolating cell and animal data to people is noted. Lastly, in addition to program objectives, improvements in certain methodologies and instruments are discussed.

A program of radiation research would play an effective role in improving the overall understanding of human cancer, and would have the potential of reducing the uncertainties in risk due to protracted, low doses of radiation. Because of the many benefits to industrialized society that are derived from the use of radiation and the devices that give rise to it, the desirability of reducing the ratio of risk to benefit via a program of research is brought out.

Appendix A

APPENDIX A

HUMAN HEALTH RISKS FROM IONIZING RADIATION - CURRENT LITERATURE

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A.1 HUMAN RADIATION HEALTH EFFECTS MODELS

There have been few large epidemiological studies of the effects of radiation on humans where the exposed populations have been followed until the end of life. As a result, there is substantial uncertainty in projection of currently available health risk data for such populations to the end of life, and certain assumptions must be made in order to make such projections. In addition, much of the available epidemiological data which has produced statistically significant results is associated with populations exposed to relatively large doses of radiation and usually at high dose rates. As a result, there is also great uncertainty in the dose response curves for the end points of interest (e.g., cancer and hereditary disorders) (NRPB, 1988, p.17).

UNSCEAR (1988) has provided a useful summary of how the risks are calculated, and some of that information is presented here for completeness. Life-time risk $[U(a_0, D)]$ to a population of age a for a dose D is calculated by the following method:

$$U(a_0, D) = \int_{a_0}^{100} V(a, D) [N(a, D)/N(a_0)] da$$

where $N(a, D)/N(a_0)$ is the probability of surviving to age a for an individual of age a_0 , taking into account the risks of radiogenic cancer and all other competing risks of mortality. Using studies of irradiated populations, the age-specific absolute excess mortality rate $[I(D)]$ and the age-specific excess relative risk per cGy $[K(D)]$ are calculated based on all the observed data.

A.1.1 Relative Risk Model

The relative risk (RR) model is a multiplicative risk model in which the ratio of the excess cancer in an exposed population to a comparable unexposed population is assumed to remain constant to the end of life after an appropriate latent period following a given dose. Therefore, the projected estimates of future cancer mortality are multiples of the natural cancer risk in the exposed population. That is,

$$R(\text{exposed}) = RR \times R(\text{unexposed}, t)$$

where RR is constant for all times $(t) > \text{latency period}$.

Since the baseline age-specific cancer rates in an unexposed population increase rapidly with advancing age, the projected (i.e., the calculated) cancer rates in the exposed population will also increase rapidly with age following exposure (BEIR III, 1980, pp. 30-31). This relationship is shown in Task 1, Figure 1.5, taken from the BEIR III report (1980).

As will be seen, when the current data are examined, this model is believed to be appropriate for projecting future risk for most tumors (BEIR V, 1990).

UNSCEAR (1988) describes the relative (multiplicative) risk model used for projection of risks as:

$$U_m(a_0, D) = K(D) \int_{a_0+L}^{a_0+L+P} C(a) [N(a, D)/N(a_0)] da$$

where L is the minimum latency period and P the plateau period following exposure at age a.

A.1.2 Absolute Risk Model (AR)

The absolute risk model (AR) is an additive model in which the expression of cancer is independent of the natural age-specific cancer following exposure. After an appropriate latent period, where no excess cancers are detectable in an exposed population, this model assumes that the cancer induction rate is constant per unit of dose for a population of a given age (BEIR III, 1980, p.30). For a given exposure, the rate is assumed constant and additive to the spontaneous cancer rate for an additional period of time following the latent period (the so-called plateau). That is:

$$R(\text{exposed}, t) = AR + R(\text{unexposed}, t)$$

where AR is the absolute excess risk for all times (t) > latency period.

This model is shown in Task 1, Figure 1.5. Prior to the BEIR V report, this model was thought to best represent the risk of radiogenic leukemia, and to possibly be appropriate for projections of cancer of the thyroid, bone and liver.

The calculational model employed for absolute (additive) risk projection was described by UNSCEAR as:

$$U_a(a_0, D) = I(D) \int_{a_0+L}^{a_0+L+P} [N(a, D)/N(a_0)] da$$

where parameters are as discussed above under the general model, L is the latency period following exposure at age a, and P is the length of the period of increased risk (plateau).

A.1.3 Biological Response Models

Generally speaking, neither human nor animal response data provide adequate information to statistically establish the shape of the biological response curve at low doses, because the sample sizes required to unequivocally do so are too large to be practical. The size of the required sample population is approximately inversely proportional to the square of the cancer excess. For example, if 1,000 animals in the exposed and control groups are required to provide adequate statistical power at 100 cGy, about 10,000,000 in each group would be required at 1 cGy, and about a billion in each group would be required at 0.1 cGy (BEIR III, 1980, p.140).

Although there are several models which purport to describe the biological response from exposure to radiation, at present only three have achieved some measure of acceptance based on theory and observed responses of human populations, and of whole animals and autonomous cell lines grown in culture. All three of the models are derivable from a generalized model having both linear

and quadratic terms, and all assume no threshold for cancer risk (even though none are statistically detectable below about 10 cGy in humans). The discussions which follow will briefly describe these models, which are shown graphically in Task 1, Figure 1.2.

A.1.3.1 Linear-Quadratic Response Model

Since cancer or mutagenesis is believed to result from changes in individual cells, biological response models are derived from basic theories of cellular damage from ionizing radiation of various qualities and quantities. The linear-quadratic response model is based on observed responses of autonomous cells to low linear energy transfer (LET) radiation such as X-rays, gamma rays, and beta particles. In general, studies of cellular responses (e.g., cell killing or transformation) have been shown to be satisfactorily fitted with an equation of the following type:

$$E = a + bD + cD^2$$

where a is the zero-dose incidence (i.e., spontaneous rate), D is dose, and b and c are empirically derived coefficients. Another correction factor (not shown here) would be required to account for the effects of cell killing at high doses, but is not relevant to the low dose, low dose rate effects of interest for this study. Classical radiobiology theory holds that b and c represent a mixture of one and two-track events within cells. Since the number of tracks depends on radiation quality, b and c would be dependent on LET. On a cellular level, modern theory holds that the quadratic function may represent misrepair of breaks (lesions) in pairs of DNA molecules (double strand breaks) in adjacent chromosomes. From a microdosimetric standpoint, high-LET radiation produces so much greater energy depositions within a cell that double breaks predominate. However, for low absorbed doses of low-LET radiation, the likelihood of two-track events becomes so low that the quadratic term becomes relatively unimportant, and the response becomes linear (BEIR III, 1980, pp. 12-23).

This model almost certainly oversimplifies the real situation in cells (e.g., it doesn't discuss the variation in sensitivity of cells as they proceed through the cell cycle, time-dependent events such as cell repair, or the effects of cell-killing at high doses on the likelihood of detecting cell transformation). As a result, these effects on the expression of cellular transformation resulting in cancer are still highly uncertain. However, for a general application to fitting epidemiological data on human populations, the general form of the linear-quadratic response model is currently believed to be adequate for fitting an empirical curve to human data and determining human risk from most types of radiation induced cancer.

A.1.3.2 Linear Response Model

The linear dose response model is used to fit observed human health effects to experience, typically following high doses, generally at high dose rates (e.g., the Japanese bomb survivors). The model is based on the assumption that there is no dose threshold for radiation health effects, so a straight line can be drawn from the zero dose incidence through the observed effects from high doses. This assumption basically means that the quadratic term in the general model is relatively unimportant, even at doses approaching lethal levels. While this assumption ignores animal and cellular data to the contrary, the statistical tests on human data to date have lacked the statistical power to reject either

the linear or the linear-quadratic models. Given this situation, and the greater ease in using the linear model to calculate risks at different doses, there is something to be said for using the simpler model. In this model, the coefficient in the aD term is simply the slope of the line fitted to the cancer risks for the largely high-dose, high-dose-rate, human data currently available.

The United Nations Scientific Committee on the Effects of Atomic Radiation report to the United Nations for 1989 (UNSCEAR, 1989) has accepted the use of the linear dose response model, but has recommended dose-rate effectiveness factors (DREF) for use at low dose-rates (less than 10 cGy per year) of low-LET radiation. The factors selected by NRPB are 2 for breast cancer and 3 for all others (see NRPB, 1988, pp. 24-25 and 60-62). This approach is similar to that employed in the Reactor Safety Study (USNRC, 1975).

A.1.3.3 Quadratic Response Model

The quadratic response model was considered by BEIR III to reasonably fit the available high dose data, and to provide a lower bound to the envelope of risks (around the linear-quadratic) it was attempting to develop for Federal guidance (BEIR III, 1980, p.142).

Acceptance of the quadratic model as a reasonable description of the risk to radiation exposure appears questionable, since, in a classical sense, it implies that single hits are unimportant in production of cancer, and all the observed effects can be explained in terms of double-tracks. While that may be true for existing high-dose, low-LET, human-risk data, the use of such models to extrapolate to the risk of low-dose, low-dose-rate exposures is scientifically questionable.

A.2 REVIEW OF CURRENT *IN VIVO* HUMAN EPIDEMIOLOGICAL DATA

Since the BEIR III report was published in 1980, a substantial revision of the dosimetry for the exposed Hiroshima and Nagasaki populations has been completed. In addition, new data from Japanese survivors and other exposed human populations have continued to accumulate. This review will focus on the results of current reevaluations of the health risks to those populations.

A.2.1 Radiation Effects Research Foundation (RERF) Findings

In December 1984, the Radiation Effects Research Foundation (RERF) received a preliminary new dosimetry system, designated DS84, which was developed by Science Applications International Corporation (SAIC), under contract to the U.S. Department of Energy (Kaul, 1983, pp.57-103 and 218-24.). This system was used to develop a set of interim dose estimates for about 60% of the exposed survivors in the Atomic bomb survivor Life Span Study (LSS) with detailed shielding histories (only 13% of exposed survivors in the LSS-E85 cohort) which were designated as I85D. The final 1986 dosimetry system was termed DS86.

The Final RERF report was published in 1987 (Roesch, 1987). Among the findings which affected the risks were several changes in the free-in-air kerma estimates. At Nagasaki, the gamma-ray kerma for DS86 was about 10-30% less than for the 1965 tentative dosimetry system (T65D), depending on ground range, while the neutron kerma was about a half to a third of the T65D. At Hiroshima, the gamma-ray kerma for DS86 was larger than T65D by about a factor of 2 to 3.5,

depending on ground range; the DS86 neutron kerma was about 10% of the T65D kerma. The rest of the change in gamma-ray kermas (factors of 1.7 to 2.9) was the result of the different methods used in the two dosimetry systems, especially for Hiroshima (Ibid, p. 18). A major factor which prevented the risk coefficients from rising even more than has been observed was the fact that the gamma-ray transmission factor for DS86 in Japanese houses was 0.53 for prompt gamma and 0.46 for delayed gamma at 1500 m versus an assumed T65D factor of 0.9. The neutron transmission factor for DS86 was 0.38 versus 0.32 for T65D. The organ dose estimates were considerably more sophisticated than for T65D, and resulted in a much higher organ transmission factor, compensating in large part for the reduced gamma-ray transmission factor for houses.

In July 1988, the RERF published the initial results of applying the new DS84 system to 12,237 survivors who were beyond 700 m from the blast hypocenters at the time the bombs exploded (ATB) (Kopecky and Preston, 1988). These interim results, which were actually prepared in September 1986, must be reviewed with care, since the RERF report by Shimizu, et al. (1987) is actually more recent. The results were intended to indicate the kinds and magnitudes of changes which might be expected to result from the adoption of the DS84 system to replace the older T65DR system initially used for the A-bomb survivors. The major changes in risk coefficients were the result of the changes between the T65D and DS84 exposure dose estimates (in general neutron doses were greatly reduced while the gamma doses were somewhat less reduced).

The interim results for the risk coefficients indicate that the I85D dose estimates support the use of a linear dose response function for both the RR and AR models for leukemia and other cancers (Kopecky and Preston, 1988, pp. 25-27). Further, assuming an RBE of 10 for neutrons, RERF found that the risk estimates based on I85D are consistently about 2.5 to 2.75 times greater than those based on T65DR (reflecting the large neutron dose reductions among Hiroshima survivors) for both the AR and RR projection models (Ibid, pp.30 and 33).

In 1987, Preston and Pierce published another RERF report on the effect of changes in the Hiroshima and Nagasaki data, using the latest dose estimates (DS86). This report, unlike previous RERF and Atomic Bomb Casualty Commission reports which used tissue kerma in air, used organ dose estimates to estimate cancer risks among A-bomb survivors. The authors warn that it would be "seriously misleading" to restrict attention to risk estimates based on kerma only, "because the changes in terms of organ doses and dose equivalents can be different from those for kerma-based risks," especially when organs (e.g., bone marrow and large intestine) receive substantial shielding by the body (Preston, 1987, p.3). This report concludes that "there is little difference between the new and the old dosimetrics based on total organ dose." However, consistent with preceding reports, for a range of RBE values for neutrons of 1 to 20, the differences in risk estimates between Hiroshima and Nagasaki (based on TD65 dose estimates) are smaller based on the new dosimetry, and are no longer significant between cities (Ibid, p.4). Although estimation of RBE is even less feasible with the new dosimetry, for an assumed RBE of 10, risk estimates for leukemia and nonleukemia are 80% and 30 % higher, respectively, than previously estimated (Ibid, p.33).

Those estimates are based on exclusion of individuals whose estimated organ doses exceeded 400 cGy, presumably to avoid inclusion of reduced risks due to cell killing or other effects (Ibid, p.32). A linear dose-response model was assumed here, even though the study identified substantial non-linearity in the

dose-response model based on estimated vs. true doses (i.e., the observed non-linearity may not be real due to imprecise dose estimates). An absolute risk model was used for leukemia, since the period of risk appears to be over, and a constant relative risk model was used to project lifetime risk for nonleukemia (Ibid, Table 13).

The November 1987 RERF report by Shimizu, et al. (1987) examined the dose-response curves for mortality for the T65D and DS86 results, and found the DS86 dose-response curve higher in both cities than the T65D response. However, the non-linear response curve observed for the T65D and disturbingly higher mortality in Hiroshima for the same dose were not present in the reassessment using the DS86 dose estimates (Ibid, pp.17-18). In addition, although responses still varied within the low dose range for DS86, "there was no statistically significant evidence of a positive effect [excess leukemia] at low doses even when the observations are distributed over finer subdivisions, i.e., 0, 0.01-0.05, 0.06-0.09, 0.10-0.19, and 0.20- 0.49 Gy" (Ibid, p.18).

The most recent RERF study by Shimizu et al. (1988) examined the cancer mortality (1950-85) based on the DS86 revised dose estimates, focusing on the appearance with time of radiation-induced cancer and the effects of such risk modifiers as age at exposure, age at time of death, sex, city, and carcinogens other than radiation (i.e., smoking). The results are summarized below:

- Excess leukemia mortality has continued to decline with time, but is still slightly but significantly elevated in Hiroshima through 1985;
- For cancers other than leukemia, excess mortality continues to increase with time in a manner consistent with the relative risk (RR) model among those greater than ten years of age when exposed;
- For those exposed as children (0-9 years), the time from exposure to death (i.e., the latent period) was shortened for all cancers except leukemia in the high dose (> 100 cGy) group, and the RR decreases with time at attained ages under 30 years, then levels off at attained ages of 40 to 49;
- The RR model was found to best estimate lifelong risk for all cancers except leukemia;
- Both relative risk and absolute risk were higher for those exposed when younger;
- For all cancers except leukemia, excess risk (RR) does not vary significantly over time;
- When adjusted for smoking, there were no sex differences for cancer, except that leukemia and breast cancer risks were higher for females;
- Increased cancer risk was observed only for leukemia and cancers of the esophagus, stomach, colon, lung, breast, ovary, urinary bladder, and multiple myeloma (unlike earlier studies, no excess thyroid cancer was detected);
- For leukemia, the linear dose-response model fits well except at the high dose range;

- The linear dose-response model shows a good fit for all solid tumors;
- The linear dose-response model for the new dosimetry results in a risk value about two times higher than the BEIR III linear model, and considerably higher than the BEIR III linear-quadratic model.

The RR and AR coefficients for those cancers found to be increased statistically above spontaneous levels were determined for Hiroshima and Nagasaki survivors combined as shown in Table A.1.

Table A.1. Summary of Statistically Significant Cancer Mortality Dose Response for all Hiroshima and Nagasaki Survivors Combined

Type of Cancer	RR at 1 Gy (90% CL) ^a	Excess Deaths per 10,000 PYGy	Attributable Risk (Percent)
Leukemia	6.21(4.83,8.12)	2.94(2.43,3.69)	58.6(48.4,69.5)
All Cancers Except Leukemia	1.41(1.32,1.51)	10.13(7.96,12.44)	8.1(6.4,10.0)
Esophagus	1.58(1.13,2.24)	0.45(0.10,0.88)	13.0(3.0,25.5)
Stomach	1.27(1.14,1.43)	2.42(1.26,3.72)	5.7(3.0,8.7)
Colon	1.85(1.39,2.45)	0.81(0.40,1.30)	16.3(8.0,26.2)
Lung	1.63(1.35,1.97)	1.68(0.97,2.49)	12.3(7.2,18.3)
Breast	2.19(1.56,3.09)	1.20(0.61,1.91)	22.1(11.3,35.0)
Ovary	2.33(1.37,3.86)	0.71(0.22,1.32)	22.3(6.9,41.4)
Urinary Tract	2.27(1.53,3.37)	0.68(0.31,1.12)	21.5(9.8,35.7)
Mult. Myeloma	3.29(1.67,6.31)	0.26(0.09,0.47)	31.8(11.0,57.6)

^anumbers in parentheses indicate 90% confidence interval.

The RR and AR risk coefficients for certain cancers for all survivors combined are summarized in Table A.2 by sex and age ATB for an assumed RBE of 10 for neutrons in the absorbed organ dose.

Table A.2. Excess Cancer Risk for RR and AR Models for Leukemia and All Other Cancers based on DS86 Calculated Organ Absorbed Dose^a by Age at Exposure and by Sex

Type of Cancer	Risk Model	Sex	Age at Exposure				
			<10	10-19	20-29	30-39	40+
Leukemia	RR	M	17.9	4.8	5.9	4.5	3.9
		F	18.8	5.0	6.2	4.7	4.0
All Others	RR	M	1.96	1.60	1.52	1.23	1.16
		F	2.92	2.20	2.04	1.45	1.32
Leukemia	AR	M	3.46	1.79	3.87	5.72	4.22
		F	2.71	0.92	2.24	1.79	2.88
All Others	AR	M	1.35	4.87	11.5	10.6	15.1
		F	3.79	6.70	12.9	13.1	17.5

^a100 cSv for RR, one million person-cSv for AR.

A.2.2 Committee on the Biological Effects of Ionizing Radiation (BEIR V Report)

In December 1989, the National Research Council's BEIR Committee (BEIR V, 1990) released its latest review of the risks of low-LET ionizing radiation, reflecting the results of the Hiroshima and Nagasaki revision of dose estimates to the Japanese A-bomb survivors as well as new human health data acquired since the 1980 BEIR III review (NRC, 1990). Because cancer risks are dependent on age at exposure, risk projections should be based on observation of cumulative risks of each age cohort throughout their lives. Unfortunately, the numbers of observed cancer sites in many age cohorts are often small or zero, making detailed risk estimates impossible at this time. Attention was focused on estimating the risk for leukemia, breast cancer, thyroid cancer, and cancers of the respiratory tract and digestive system, where the number of excess cases are statistically substantial. However, to estimate the total risk of cancer mortality from all cancers, the Committee also modeled "other" cancers.

While the Hiroshima and Nagasaki Life Span Study (LSS) data were relied on most heavily, other studies were also used to estimate incidence or mortality risks for leukemia and specific sites such as breast and thyroid. The Japanese LSS data consisted of 8,714 records that were stratified by sex, city, exposure groups, and five-year intervals of attained age, age at exposure, and time since exposure. Most of the analyses used a reduced data set of 3,399 records obtained by reducing the number of groups by attained age. Survivor exposures were stratified into ten groups and organ doses using the DS86 dosimetry system by multiplying the neutron and gamma kermas by city-specific and age-specific body transmission factors. Because the neutrons doses are too small with the new DS86 dosimetry to permit estimates of neutron RBE, the Committee assumed an RBE of 20 (although an RBE of 10 leads to essentially the same results). All records with

organ doses greater than 400 cSv were eliminated from the analysis to avoid effects of cell killing at high doses (i.e., the response curve levels off at these large doses). Also, eliminated were records of cancer mortality at attained ages greater than 75 years because of the "lesser reliability of death certificate information." The only other cohort study that provided data on all cancers was the ankylosing spondylitis series (ASS), but that study provides no dose estimates for individuals (only average organ doses for the entire group), and there was no control population (the national rates were used to derive the expected number of cases). Because the numbers of cancers observed were relatively small, and the risk of colon cancer may be causally related with the occurrence of ankylosing spondylitis, only the risk of leukemia and "all other cancers" (except colon cancer) were estimated.

Leukemia

The Committee concluded that the risk of leukemia was best fit with a relative risk model, and a linear-quadratic dose response. A minimum latency of 2 years was assumed, and age specific risks for those exposed before age 20, and those exposed later in life. However, the risks within each of the two age groups does not appear different, rather the time pattern for onset of leukemia is different.

Cancers Other than Leukemia

Cancers other than leukemia were found to be best fit by a relative risk model with a family of linear dose-response equations. All cancers occurring less than 10 years after exposure were excluded (i.e., a 10-year latent period was assumed).

Respiratory Cancer

In the case of respiratory cancer, the relative risk model declines with time after exposure (about a factor of 5 over the period 10 to 30 years post-exposure), although the fit of a constant relative risk model to the data was not statistically different from the Committee's chosen model. However, the preferred model which adjusts for time after exposure provided the greatest improvement in describing the data, and was consistent with the decreasing relative risk observed for the ASS data. In addition, modest improvement was observed if a parameter for sex was added. No improvement for including a parameter for age at exposure was observed.

Breast Cancer

The breast cancer model found that the best fit was obtained by including a variable for age-at-exposure less than 16 (reflecting changes in hormonal and reproductive status). The greatest risk was observed for women 15-20 years of age at exposure, and are very low for women exposed at ages greater than 40. It was found that in addition to declining risks with age at exposure, risks also decline with time after exposure (i.e., not a constant relative risk).

Details of the modeling used for breast cancer risk made use of mortality data from the Canadian TB Fluoroscopy Study as well as from a subcohort of the LSS for which DS86 doses are available (1950 - 1980). Incidence data from the same LSS subcohort used for mortality risk were also used along with incidence data from the New York Acute Postpartum Mastitis study and Massachusetts TB Fluoroscopy Study. All data on the first five-years of follow-up were omitted

along with data on women with the highest exposures (presumably to avoid data associated with cell-killing).

The LSS data indicated an excess relative risk about 50% greater than that in the two U.S. cohort studies, but the difference was not statistically significant. However, the LSS data for the absolute risk model indicated a risk that was about half that of the U.S. cohorts, and the difference was statistically significant.

Digestion System Cancers

For digestive cancers, the most significant finding was a factor of 7 greater risk for those exposed under the age of 30, although there was no evidence of decreasing risk with time after exposure.

Other Cancers

The remaining cancers contributed significantly to the overall cancer risk, but finer subdivision into organs did not provide enough cases for modeling individual sites. The group of "other cancers" was reasonably fit by a simple linear model with declining risk for survivors exposed beyond age 10, and no effects for sex or time after exposure.

A.2.2.1 Risk Assessment, BEIR V Report

The Committee used the 1980 U.S. vital statistics as the baseline data for cancer mortality, and then employed standard lifetable methods to provide estimates of cancer risk for three patterns of exposure:

- Instantaneous exposure (like the A-bomb survivors experienced) causing a 10 cSv dose equivalent to all body organs to population age cohorts in 10 year intervals, taking the population-weighted average dose, weighted by the probability of surviving to a specified age in an exposed stationary population;
- Continuous lifetime exposure of 0.1 cSv to all body organs per year of life;
- Continuous exposure of 110 cSv/year to all body organs from age 18 to 65.

However, as noted by the Committee the method of lifetime excess risk estimation differs from BEIR III (1980) and UNSCEAR (1977 and 1988). These estimates are simply the differences in separate lifetime risks for exposed and unexposed populations (including competing risks of mortality from other cancer). It was estimated that the BEIR V method results in about 0.8 the values from previous estimates (BEIR V, 1990, p.174).

The results of the BEIR V assessment of "excess" cancer mortality for these scenarios is summarized below in Table A.3.

The 90% confidence levels refer only to the statistical uncertainty due to random error in sampling variation, which is thought to represent the greatest source of uncertainty. The Committee also recognized there was also uncertainty in how well the Committee's preferred models actually represented the data, as

Table A.3. Excess Cancer Mortality per 100,000 Exposed Persons

	Male			Female		
	<u>Total</u>	<u>Nonleukemia</u>	<u>Leukemia</u>	<u>Total</u>	<u>Nonleukemia</u>	<u>Leukemia</u>
Single 10 cSv Exposure	770	660	110	810	730	80
90% Conf. Limits	540-1,240	420-1,040	50-280	630-1,160	550-1,020	30-190
Continuous Lifetime Exposure to 0.1 cSv per year	520	450	70	600	540	60
90% Conf. Limits	410-980	320-830	20-260	500-930	430-800	20-200
Cont. Exposure to 1 cSv per year; Ages 18 to 65	2,880	2,480	400	3,070	2,760	310
90% Conf. Limits	2,150-5,460	1,670-4,560	130-1,160	2,510-4,580	2,120-4,190	110-910

well various potential biases in the data themselves. To better evaluate overall uncertainty, the committee undertook Monte Carlo simulations in which parameter vectors for each cancer site were randomly sampled from multivariate normal distributions with means and covariate matrices given by their maximum likelihood estimates. Where components showed significant skewness, they were adjusted by multiplying the deviations from the means of the sampled value by the ratio of the likelihood-based to asymptotic confidence intervals for the corresponding 90% upper or lower tail (depending on which direction they were skewed). Then 1,000 Monte Carlo simulations were done for each sex for leukemias and all solid cancers combined, for the three exposure scenarios. Figures 4-1 and 4-2 of BEIR V show the results of these simulations, and indicate that the overall uncertainty is somewhat larger than that considered for random sampling error alone. For example, for leukemia mortality, 50% of the trials for males indicated their excess mortality fell between 60 and 135, while 50% of the females fell between 55 and 115 (versus 50 - 280 in the 90% confidence limits based on random bias for males, and 30 - 190 for females).

Since all of the above scenarios are for low dose rates, and the LSS and ASS data are all for high dose rates, the Committee assumed a dose rate effectiveness factor (DREFs) of 2 for leukemia, but one for all other cancers. This represents a major departure from all previous studies, including BEIR III, which have all assumed that dose-rate effects occur for all cancers, and results in a significant increase in lifetime risk estimates beyond those which resulted from

the revised bomb survivor dosimetry (which might have caused no more than a factor of 2 increase in the BEIR III risk estimates). The Committee's explanation for a DREF of one for all non-leukemias is that an RBE of 1 for gamma non-leukemias is that an RBE of 1 for gamma rays (such as experienced by the bomb survivors) may be high by a factor of 2 relative to observations in the laboratory where X-rays were used. Since the Committee suggests that a DREF of 2 would be approximately offset by the difference in actual vs. assumed RBE, they elected not to employ a DREF for the LSS data. That, however, does not acknowledge many observed DREFs of 2 to 5 for animal studies using either gamma or X-rays, nor does it explain why they assumed a DREF of 2 for leukemia.

As shown in Table A.4, contrary to the BEIR V press release and the executive summary at the beginning of BEIR V, the results of the BEIR III and V studies are substantially different. It should be noted that the large range in ratios minimize the range, since the BEIR III values are only for the BEIR III preferred LQ-L models, and do not include either the L-L or Q-L models. For example, if the BEIR III Q-L model were compared with the preferred BEIR V model for a single 10 cGy instantaneous exposure, the BEIR III model would project about 9.5 (absolute risk) to 28 (relative risk) cancers per million person-cSv while the BEIR V preferred model would predict about 790 cancers per million person-cSv. That would be about 28 (relative risk) to 83 (absolute risk) times higher than BEIR III. A similar comparison with the BEIR III L-L models, however, would only yield a range of ratios of about 1.6 (relative risk) to 4.7 (absolute risk). Thus, the overall range of ratios between the BEIR V preferred model and all the BEIR III models would be from about 1.6 to 83.

Table A.4. Comparison of Lifetime Excess Cancer Risk Estimates from the BEIR III (LQ-L) and BEIR V Reports

	Continuous Lifetime Exposure, 0.1 cSv Per Year (deaths/100,000)		Instantaneous Exposure, 10 cSv (deaths/100,000)	
	<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>
<u>Leukemia</u>				
BEIR III	15.9	12.1	27.4	18.6
BEIR V	70	60	110	80
Ratio (V:III)	4.4	5.0	4.0	4.3
<u>Nonleukemia</u>				
BEIR III				
Absolute	24.6	42.4	42.1	65.2
Relative	92.9	118.5	192	213
BEIR V	450	540	660	730
Ratio (V:III)	4.8-18.3	4.6-12.7	3.4-15.7	3.4-11.2

The Committee concluded that the assumption of a constant additive excess risk is no longer tenable in the face of the current data, and that the risk estimates in BEIR III are too low (especially those based on an absolute risk

model, but also those based on the relative, linear-quadratic model). The Committee's risk estimates are not greatly different from the upper bound estimates of the United Nations Scientific Committee on the Effects of Atomic Radiation 1988 Report to be discussed in the following section.

The Committee's findings are almost certain to create controversy for years to come, although, as discussed in Section 1.2.1.3 of this report, much of the animal data available also supports the use of a relative risk model for cancer induction. The ultimate outcome of these changes is quite likely to have substantial impact on public pressures to reduce still further the regulatory limits for occupational and public radiation exposures.

A.2.2.2 The Committee's Use of A-bomb Survivor RERF LSS Data

Some of Committee's use of the LSS data is presented above, and only important specific information not already discussed is presented in this section.

One important change in past BEIR modeling came about from studies of death certificates by specific cause; these studies showed that some errors in site certification were numerous. This was the case for cancers of the liver and pancreas which were often assigned to stomach cancer on the death certificates. As a result, the Committee modeled all cancers of the digestive system as a group.

The Committee found the use of relative risk models (vs additive, absolute risk models) not only provided a better fit of the data, but also had an additional advantage: relative risks are less affected by losses of cause of death assignments as the result of errors in certification by site. On the other hand, the Committee noted that absolute risks are greatly affected by losses due to erroneous certification. Further, the Committee found that RERF autopsy data shows that losses from erroneous certificates are independent of survivor dose estimates. The Committee also took exception to the simpler notions of absolute and relative risk which have been used by other major reviews, and chose to estimate risk by inclusion of "several explanatory variables in the excess risk term." Specifically, the committee chose to use the relative risk formulation with a stratified or nonparametrically estimated background (i.e., spontaneous cancer rate). The Committee compared their preferred model (stratified background) with an absolute risk model (modeled background) and a relative risk model (modeled background) and found the three models were in reasonable agreement (within about a factor of 2) and within the statistical confidence intervals of the preferred models, which differentiate between cancer types (detailed comparisons are provided in BEIR V, 1990, Table 4D-3). In addition, the Committee found the preferred models fitted the models as well as the alternatives, and with fewer terms.

The Committee also noted that due to the limited data on cancer (especially non-leukemia) among those survivors exposed as children, estimates for the young represent a model dependent extrapolation from exposed adult data. Thus, such estimates are more imprecise, and more years of follow-up are needed to provide the experience data.

A.2.2.3 The Committee's Handling of Uncertainty

Although the previous discussions have provided general information on treatment of uncertainty, especially with regard to random sampling errors, the BEIR V Committee has provided a useful discussion of other factors, and how the uncertainty associated with random error can be applied to calculation of probability of causation. BEIR V noted that the effect of applying the Japanese cancer mortality data to other populations (e.g., caucasian or african) results in an added source of uncertainty, since baseline cancer rates for specific sites vary substantially between different populations. The Committee concluded there is not yet any objective method to estimate such uncertainties, and a consensus of expert opinion was used.

The procedure employed involved expressing the uncertainties on a scale commensurate with ordinary statistical measures of variability; i.e., as the Geometric Standard Deviation (GSD). GSDs represent the range of uncertainty associated with a value. For example, if the uncertainty is 20%, the range would be the value itself divided by 1.2 to the value multiplied by 1.2. As a further example, the Committee pointed out that if the excess relative risk were estimated to be 0.3 per Gy with an uncertainty of 1.4 ($\sigma = 0.336$, and $e^{0.336} = 1.4$), 68% of the value lies in the range of 0.3/1.4 (or 0.21) to 0.3x1.4 (or 0.42). This was referred to as the "68% credibility interval" rather than confidence interval, since the values were derived through a combination of calculation and expert judgement. The basic assumption is that the error in the final estimate of risk is distributed lognormally (i.e., the logarithms of the errors are normally distributed). The Committee argued that since the logarithm of the total error is the sum of the logarithms of the individual components of error, and the distribution of a sum of variable is known to be approximately normal, it is unlikely that the assumption of lognormal distribution is "seriously wrong" (p. 221). Thus, using the previous example of $\sigma = 0.336$ at the 68% credibility interval, the range for the 90% credibility interval would be $1.2/\exp(1.645 \times 0.336)$ to $1.2 \times \exp(1.645 \times 0.336)$, or 0.173 to 0.521. Similarly, the range for the 95% credibility interval would be derived at by using two sigma, or $\exp(2.0 \times 0.336)$.

The value of the overall error was calculated as the square root of the sum of the squares of the standard deviations of the logarithms of the (assumed) independent, individual sources of error. The results of the Committee's estimates of the GSDs for several potential sources of uncertainty are summarized below:

<u>Source of Uncertainty</u>	<u>GSD</u>
1. Model mispecification	
Males	1.16
Females	1.08
2. Population differences	1.20
3. Dosimetry system (DS86)	1.10
4. Sex (leukemia and other cancers)	1.10

The Committee's estimates of the total GSDs for various cancers for males and females are summarized below:

TOTAL GSD

(All Except Leukemia and "Other")

Males = 1.29
Females = 1.25

"Other" Cancers and Leukemia

Males = 1.31
Females = 1.27

Estimates of "excess" relative risk from a 10 cGy acute dose and their GSDs associated with random sampling error (Table 4F-1, pp. 225 - 226) are reproduced in Table A.5.

Similar tabulations are presented in BEIR V for breast cancer incidence, but are not replicated here. The GSDs are very similar to those for leukemia and other cancers, ranging from about 1.24 to 2.99. The use of these data was demonstrated in BEIR V for breast cancer incidence, and are demonstrated below for leukemia based on the risk values and GSD presented in BEIR V). For example, consider a woman aged 25, exposed to 10 cSv; the excess relative risk according to BEIR V is 0.057 and the GSD is 1.54 for 25 years after exposure (age 50). The excess relative risk following a 2 cSv dose would be $(2/10) \times 0.057 = 0.0114$, and the 68% "credibility interval" would be $0.0114/1.54$ to 0.0114×1.54 , or 0.0074 to 0.0176 (0.74% to 1.76%). The probability of causation (PC) would have a range of $0.0074/1.0074$ to $0.0176/1.0176$, (0.74% to 1.73%), with a best estimate of $0.0114/1.0114$ or 1.13%. According to BEIR V, for a person aged 1 to 20 receiving 10 cSv, the leukemia mortality risk 15 years post-exposure is 3.637, and the GSD is 2.80. For a second example, consider the case of a 1 year old child exposed to 100 cSv (total from several examinations) from essentially whole body fluoroscopy (1940 technology), the excess relative risk can be calculated as $(100/10) \times 3.637 = 36.37$. The expected "68% Credibility Interval" would then be estimated to be $36.37/3.637$ to 36.37×3.637 , or 10 to 132. The PC range would be $10/11$ to $132/133$ or 90.9% to 99.2%, with a best estimate of $36.37/37.37$, or 97.3%.¹

The Committee's lifetime estimates of excess cancer mortality by time at exposure and site per million person-cSv (100,000 people exposed to 10 cSv each) are shown in Table A.6.

¹ This example represents an estimate of the PC for the sister of the author, who died of leukemia at age 10 in 1951 following several fluoroscopic examinations for a congenital birth defect during her first year of life.

Table A.5. Estimates of Excess Relative Cancer Mortality Risk from 10 cGy Acute Dose and Their GSDs due to Sampling Variation

Cancer Type	Age at Exposure	Time After Exposure	Risk	GSD
Leukemia	≤20	<15	3.637	2.80
		16 to 25	0.291	2.53
		≥26	0.027	3.32
	>21	≤25	0.287	1.83
		26 to 30	0.139	2.52
		≥31	0.0027	3.32
Breast Cancer	5-15	15	0.418	1.90
		25	0.427	1.60
		35	0.230	1.57
		45	0.105	1.89
	25	15	0.056	1.77
		25	0.057	1.54
		35	0.031	1.60
		45	0.014	1.99
	35	15	0.030	1.90
		25	0.030	1.76
		35	0.016	1.85
	45	15	0.016	2.31
		25	0.016	2.25
	55	15	0.008	2.99
Respiratory Cancer	All Ages	15	Males: 0.096	1.59
			Females: 0.196	1.47
		25	Males: 0.046	2.03
			Females: 0.094	1.76
		35	Males: 0.028	2.63
			Females: 0.058	2.27
		45	Males: 0.020	3.23
			Females: 0.040	2.80
Digestive Cancer	All Ages	All Times	Males: 0.081	1.50
			Males: 0.081	1.50
			Females: 0.196	1.47
		>10	Males: 0.011	1.88
			Females: 0.019	1.77
"Other Cancers"	5	All times	0.123	1.53
	15	>10	0.097	1.40
	25		0.061	1.31
	35		0.038	1.45
	45		0.024	1.75
	55		0.015	2.17
	65		0.009	2.71

Table A.6. Estimates of Excess Cancer Mortality by Age at Exposure and Site per Million Person-cSv of Low-LET Irradiation*

Age at
Exposure Total Leukemia Nonleukemia Respiratory Digestive Breast Other

MALES

5	1,276	111	1,165	17	361	-	787
15	1,144	109	1,035	54	369	-	612
25	921	36	885	124	389	-	372
35	566	62	504	243	28	-	233
45	600	108	492	353	22	-	117
55	616	166	450	393	15	-	42
65	481	191	290	272	11	-	7
75	258	165	93	90	5	-	-
85	110	96	14	17	-	-	-
Average:	770	110	660	190	170	-	300

FEMALES

5	1,532	75	1,457	48	655	129	625
15	1,566	72	1,494	70	653	295	476
25	1,178	29	1,149	125	679	52	293
35	557	46	511	208	73	43	187
45	541	73	468	277	71	20	100
55	505	117	388	273	64	6	45
65	386	146	240	172	52	-	16
75	227	127	100	72	26	-	3
85	90	73	17	15	4	-	-
Average:	810	80	730	150	290	70	220

*These estimates are for 10 cSv exposures to a population of 100,000 persons of that age chart; the averages are weighted for the age distribution in a stationary population with U.S. mortality rates.

A.2.2.4 BEIR V Estimates of Cancer Risk at Specific Sites

Leukemia

Although the Committee found marked differences in the age distributions of the various types of leukemias, modeling of the various leukemias was not possible due to the limitations of the current data. As a result, the Committee chose to simplify the risk model by combining all hematologic types of leukemia except chronic lymphocytic leukemia (which has been shown not to be a radiogenic form). For both the LSS and ASS data, the Committee found the data could be fitted approximately as well with either an absolute or relative risk model. However, the Committee found the relative risk model consistently "more

parsimonious" with the data (i.e., the relative risk model required less modifying effects and simpler modeling to achieve a satisfactory fit).

The Committee's preferred model for the ASS data is a relative risk model with decreasing effect with time after exposure. However, the addition of a modifying factor for effect of age at exposure (≤ 20 or >20 years) to the LSS data significantly improved the fit. The ASS data had no cohort under the age of 20, and the use of this modifying factor could not be tested with that data. Dose response in the LSS data was also significantly improved by the addition of a quadratic term in dose (for gamma component only). The dose at which the linear and quadratic components were equal (i.e., the "cross-over dose") was estimated to be about 90 cGy, although the uncertainty is very large. Similarly, the committee noted the dose reduction factor (DREF; the ratio of the slopes of the pure linear and linear-quadratic models) was estimated to be 2, but with large uncertainty. The Committee argued that the DREF, however, is already incorporated into the leukemia model, since it is linear-quadratic which contains "an implicit dose-rate effect (i.e., the quadratic component "vanishes" at low doses, leaving only the linear component which "is generally taken to reflect one-hit kinetics).

The preferred model was based on the LSS data, which do not reflect information prior to 5 years after exposure (the ASS excess risk data had already peaked by that time). The Committee "corrected" the LSS data by extrapolating to 2 years the excess relative risk observed for the 5- to 10- year post-exposure period, resulting in an increase of about 15% in the lifetime risks. However, since the excess relative risk observed for the ASS data in the first 5 years represented about 35% of the total lifetime risk, the committee's estimate may slightly underestimate actual risk.

The Committee concluded that while the age at exposure is an important modifier of risk, there was no evidence to support the earlier belief that those exposed before the age of 10 had a greater risk than those exposed between the ages of 10 and 20.

Breast

The Committee fit various models to all of the existing breast cancer data including the LSS (151 deaths observed through 1985 and 367 cases observed through 1980, for which DS86 dose estimates are available), the Canadian Tuberculosis Fluoroscopy (CAN-TB) Study (473 deaths to date), the New York Acute Postpartum Mastitis (NY-APM) Study (118 cases), and the Massachusetts Tuberculosis Fluoroscopy (MASS-TB) cohort (65 cases). Because there were no observed cases of breast cancer for women less than 25 years of age, risk in the 0-24 year age group was excluded, even though it made little difference in the results of the risk modeling. Excluded from the study were women who received greater than 400 cGy in the LSS and MASS-TB cohorts, and 650 cGy in the NY-APM cohort. The committee did not explain the rationale for the different cutoffs, but presumably it reflects the limitations of the dose estimates rather than estimates of risk. Also excluded were primary cancers of the second breast.

The Committee's preferred linear, relative risk model varies with age at exposure and time after exposure. The Committee selected the relative risk projection for incidence because the relative risk for breast cancer among the Japanese survivors and the U.S. cohorts did not differ significantly, while the

absolute risks among the Japanese cohort were significantly lower than those among the U.S. cohorts.

The choice between relative and absolute risk models for mortality data was ~~mx~~ a difficult. The CAN-TB cohort risk per unit dose for women treated in Nova Scotia was about 6 times greater than that for women treated in other provinces, and that difference was not attributable to non-linearities in the dose response. Since the difference could not be explained, the Committee elected not to use the Nova Scotia data. However, in Nova Scotia, women faced the X-ray beam and therefore their breasts got higher doses (and dose rates) than in other provinces where their backs were to the beam [this data could be possibly be explained by a DREF, but that would be inconsistent with the Committee's conclusion no dose-rate effect was observed in other cohorts]. The Committee found that although the relative mortality risk was about three times greater than all the non-Nova Scotia data, the difference was not statistically significant. Further, the absolute risks for the LSS and CAN-TB cohorts were about the same. The Committee elected to combine the LSS and non-Nova Scotia CAN-TB data, and ignore the Nova Scotia data [even though the relative risks of the LSS and Nova Scotia data were very similar, both were delivered at relatively high dose rates, and this could be construed as evidence of a dose-rate effectiveness factor for the LSS and non-Nova Scotia cohorts]. The Committee noted that women in the LSS and NY-APM cohorts received acute exposures, whereas the women in both TB cohorts received highly fractionated exposures, [but all at high dose rates] usually over a period of years. Yet the Committee found no statistically significant evidence of a reduced risk per unit of dose between the LSS and NY-APM cohorts (for incidence or mortality).

Excess relative mortality risk for the 10-14 age group was found to be significantly greater than that for older women; relative risk for those under 10 years of age at exposure was somewhat lower than the 10-14 cohort, but not statistically so. Therefore, the Committee combined ages <10 with the 10-14 age cohort. The Committee's preferred model also allows increased risk under age 15, and for observed decreases in relative risk with increasing age-at-exposure beyond age 15. Mortality was modeled as a quadratic in log time (i.e., non-linear model). Although there was no evidence that the temporal pattern was affected by dose or age-at-exposure, the Committee noted that the tests for such affects lacked statistical power. The models assume there is no excess risk during the first five years after exposure and that there is no excess risk for women under 25 years of age, but there was no evidence of any significant increased risk for at least 10 years post exposure.

The youngest women in the RERF (LSS) cohort are just reaching the age where breast cancer would be expected to begin to show an excess risk, and additional follow-up will be required to permit more precise estimates of risk in that group (<10 years of age).

Breast cancer latency is dependent on whether the female is a child or an adult at time of irradiation, since the appearance of tumors is dependent on secretion of the pituitary hormone, prolactin. In rats, the time between irradiation and elevation of prolactin levels can be extended from a few days to as long as a year post exposure without changing either the time from increased prolactin levels to appearance of tumors or in the final tumor incidence. Latency in rats has also been shown to be inversely related to dose, and may be a function of the number of radiogenically initiated cells. Rat experiments with clonal irradiated cells (multicellular glandular units) indicates that a tumor

will arise for about each 240-300 of the clonogens when transplanted into unirradiated rats with prolactin (a peptide secreted by the anterior pituitary gland) and glucocorticoid (a gonadal steroid) deficiencies. The Committee concluded that women with normally functioning endocrine systems do not show any dose-related reduction in latency, but that the effects of hormones on mammary cells greatly affect the risk of breast cancer, even in unirradiated women. For example, women who have lactated have a lower risk of breast cancer, especially premenopausal cancer.

Experiments with rats have also shown the dose response is best fit with a linear model, and that there is little effect from dose fractionation or protraction over a wide range of exposures (28-400 cGy) when the experiments were terminated at 10-12 months. The only human data which show a linear-quadratic dose-response relationship is the CAN-TB cohort (with the Nova Scotia subcohort included).

In summary, the Committee concluded the following:

- Induction of breast cancer is hormonally mediated, and hormonal status is critical for radiation induced breast cancer;
- The dose-response relationship is linear;
- There is little evidence of a reduction in risk with dose fractionation;
- There is no evidence of increased risk of radiation-induced breast cancer before the age of 25;
- Risk is highest for women exposed before the age of 20, suggesting that the onset of puberty corresponds with a period of increase risk;
- There is little evidence of increased risk for women exposed after age 40;
- Occurrence of pregnancy and lactation before age 20 may reduce the risk of radiation-induced cancer;
- Human data do not show any relationship between dose and latency, and latency is at least 10 years regardless of age at exposure.

Lung

The Committee identified three sources of human epidemiologic data on the radiation induced human lung cancer:

- The A-bomb survivors (LSS) cohort;
- The ankylosing spondylitis (ASS) cohort;
- Uranium miners and other underground miners exposed to high LET radiation from short-lived radon daughters.

The LSS cohort for the period 1950-1985 (76,000 survivors) as a function of the DS86 dosimetry indicated that the relative risk declined somewhat with time for all age cohorts. The relative mortality risk was estimated to be 1.63 (90%

confidence interval, 1.35-1.97) at 100 cGy; the absolute excess mortality risk was estimated to be 1.68 deaths per million person-year-cGy (90% confidence interval, 0.97-2.49).

The ASS cohort does not include estimates of individual doses, but average doses to the lungs and primary bronchi were recently estimated to be 179 cGy and 677 cGy, respectively. However, the Committee did not report an average lung cancer mortality risk for this cohort.

The Committee noted the BEIR IV estimate of 350 deaths per million person WLM based primarily on underground miner data following exposure to short-lived radon progeny, but preferred to base its model on the a-bomb survivor data (LSS cohort). Noting that there are data suggesting a multiplicative effect on lung cancer resulting from radon progeny, the Committee found such data equivocal, and of uncertain relevance to the effects of low-LET irradiation. Since the reliability of lung cancer deaths (death certificates) declines rapidly with age over 75 years, the Committee discarded that data. Further, since those exposed as children are still too young to provide reliable information, the Committee also excluded them from their model. The Committee's preferred model is a linear dose response, relative risk model, even though the relative and absolute risk models provided almost identical fits to the data. Little effect of age at exposure was noted, although (as mentioned earlier) the relative risk declined with time after exposure. As a result, the Committee included a modifying effect for time after exposure in its model.

Stomach

The Committee concluded that the best evidence for risk of stomach cancer comes from the LSS cohort, although studies of patients irradiated for peptic ulcer at the University of Chicago and women irradiated for cancer of the cervix, as well as animal studies, all show associations between exposure and risk of stomach cancer. The LSS cohort data indicate an excess relative risk of 0.23 per Gy (kerma), and an average excess absolute risk of 2.09 deaths per million person-yr-cGy. That is the single largest excess for specific non-leukemia cancer sites. The University of Chicago data indicate a relative risk of 3.7 among patients treated with an average organ dose of 1,600-1,700 cGy. The corresponding absolute excess risk was 5.5 per million person-cGy, based on a life table analysis.

Early results of the women treated for cancer of the cervix were not quantitatively consistent in terms of risk per unit of dose. For example, the stomachs of these women received organ doses (about 2,000 cGy) that would have been expected to produce about 60 excess stomach cancers (based on the A-bomb survivors and other current data), but in fact only 3 were observed. Recent studies of these patients, however, have shown that when a subset of the original study cohort made up of women with second primary tumors were compared with their matched controls, an exposure of several Gy resulted in a statistically significant relative risk of 2.1.

A similar problem with the early results from ankylosing spondylitis treated with radiation has now apparently been resolved and the results are generally consistent with other data in terms of excess lifetime risk, although the time from treatment to appearance appears to be somewhat different.

The Committee concludes that its estimate of relative mortality risk (1.19 per Gy) is appropriate, although the current data do not permit a precise dose-incidence relationship.

Thyroid

Based on its review of the available data on risk of thyroid cancer among exposed populations, the Committee made the following generalizations:

- Risk is greater for those exposed as children, although tumors generally do not appear until after puberty;
- Females are two to three times more susceptible than males to either radiogenic or spontaneous thyroid cancer;
- Radiogenic thyroid cancer is often preceded or accompanied by benign thyroid nodules, and the frequency of simple goiter or hypothyroidism is also increased from large doses of radiation when young;
- Radiogenic carcinomas of the thyroid are generally papillary growths, with few of follicular or mixed histopathology;
- The expression of thyroid cancer is dependent on hormonal stimulation; therefore, any condition which leads to a sustained increase in the levels of thyroid-stimulating hormone (TSH) increases the risk of thyroid cancer.

The Committee concluded that because of suspected bias in diagnosis of thyroid cancer among the LSS cohort, the A-bomb survivor could not be used. With that remarkable decision, the Committee was left with only cancer risk data from patients irradiated as children. The childhood data included those exposed to scalp irradiation for treatment of ringworm of the scalp (Tinea Capitis Study) in Israel, and the Rochester Thymus Study. Only cases appearing beyond 5 years post-exposure were analyzed (the Committee estimated a minimum 20 year latency period). All Committee risk estimates for adults are based "solely on extrapolations from the childhood exposures."

The 10,834 children (ages 0-15 years) irradiated in Israel resulted in 39 cases of cancer compared with 16 cases among 16,226 nonirradiated controls. However, the background rate was about twice as high as observed in the other studies. The relative risk per Gy at age 40 for Israeli children ranged from 8.3 for those exposed at ages 5-15 to 23.6 for those exposed at ages 0-4. Excess absolute risk for the same groups ranged from 2.3 to 25.5 per million person-yr-cGy. The relative risk per Gy at age 40 for non-Israeli children ranged from 23.1 to 68.7 for those exposed at 5- 15 years of age and 0-4 years of age, respectively. Absolute risk for the same groups ranged from 6.7 to 75.7 per million person-yr-cGy.

The 2,652 infants (less than one year old) irradiated for enlarged thymus developed 37 cancers as compared with only one case in the 4,823 nonirradiated controls. Relative risk per Gy at age 40 for the Rochester thymus patients (average age at exposure was 0.5 yr) ranged from 6.7 to 19.2, while the absolute risk ranged from 0.6 to 1.8 per million person-yr-cGy.

Both the Israeli and Rochester cohorts exhibited a linear dose-response function, even though the risk estimates per unit dose were significantly different. Factors which contributed to the differences included cohort effects (e.g., racial and ethnic differences may have contributed to the increased relative risk estimates for Israeli and non-Israeli children relative to the infants irradiated in Rochester), sex (females were three times more sensitive based on absolute risk, but there were no sex differences for relative risk), age at exposure (risk for Israeli and non-Israeli children were about three times higher for children exposed before age 4), and time since exposure (Israeli relative risk data indicates that risk was proportional to the time since exposure raised to the 1.4 power; however, the Rochester indicated the observed effects are well described by a constant-excess-risk model, while the relative risk model declined with time and was proportional to time raised to the -2.8 power). Another effect was ethnic origin, although the effect appears to originate from life-style rather than genetic differences. The Committee speculated that the westernization of Israelis may account for the reason their excess cancer risk was closer to that of the Rochester cohort.

In view of the complications, the Committee made projections of lifetime thyroid cancer risk with a relative risk model based on Israeli-born children who were over 5 years old at time of irradiation. This model predicts a relative risk of 8.3 for all ages per Gy, with a "likelihood based" 95% confidence interval of 2 to 31 per Gy. On the basis of unpublished data on A-bomb survivors made available by the Radiation Effects Research Foundation (RERF), the Committee concluded that the risk of radiogenic cancer in adults is, at most, one-half that in children.

The Committee also considered the comparative differences between externally deposited radionuclides and the effects of external radiation discussed above. Comparisons of effects are complicated by dosimetry and biological factors. The larger the thyroid gland, the greater the absorbed dose (primarily from beta radiation) from I-131 depositions. However, since efficiency of iodide uptake as well as hormone secretion differs over time among thyroid follicles (which typically contain about 90% of all the iodine in the thyroid), relative dose distribution varies from follicle to follicle over time. A large scale rat experiment, however, showed no significant difference in risk per unit dose between X-rays and I-131, and the risk per cGy was 1.9×10^{-4} which compared well with the estimated human lifetime risk from exposure to X-rays.

The Committee warned that data from patients treated with radioiodine for thyrotoxicosis may not be directly applicable to normal populations, since the doses were very large (i.e., extensive cell death) and the incidence of thyroid cancer among patients with thyrotoxicosis may be as much as 10 times higher than among the general population.

After reviewing the current literature, the Committee concluded that:

- Radiogenic thyroid cancer arises exclusively from the follicular epithelium, and has about a 10% mortality rate;
- There are major differences in background thyroid cancer rates in different populations which appear to be related, at least in part, to differences in life-style;

- Females are about 3 times as susceptible to radiogenic and background cancer as males;
- Excess risk is greatest among children irradiated during their first five years of life, and risk of radiogenic cancer among adults is half or less than that of children;
- Although the data are best fit by an absolute risk model that includes allowance for cohort effects, latency, age at exposure, and sex, the Committee preferred a relative risk model because of the strong dependence of risk on background incidence; the preferred model yields a risk (based on Israeli-born children who were exposed after age 5) of 8.3 per Gy for both sexes;
- The risk ratio for I-131 vs. X-rays has been estimated as 0.66, but the 95% confidence interval ranges from 0.14 to 3.15;
- Progression of thyroid cancer from initiated cells is strongly dependent on hormone balance.

Esophagus

The Committee did not make its own estimate of the risk of esophageal cancer, but reviewed and apparently accepted the latest RERF estimates of relative risk (1.58/Gy), and also found other study results (e.g., ankylosing spondylitis and women treated for cancer of the cervix) consistent with the RERF relative risk estimates.

Small Intestine

The Committee reviewed the current data and found that there was some evidence of an increased risk following irradiation, but the risk was small and not quantifiable.

Colon and Rectum

The Committee reviewed the current data and found that there was an increased risk of mortality from cancer of the colon and rectum from large doses of radiation, but noted that the dose-response and risks are highly uncertain. It concluded that the A-bomb survivor colon cancer data indicated a relative mortality risk of 1.85 per Gy, or 0.81 excess (absolute) deaths per million person-yr-cGy, and the latent period was at least 15 years.

Liver

While most of the data regarding the risk of liver cancer comes from exposure to alpha-emitting, internally-deposited radionuclides (e.g., thorium), the Committee also reviewed the current LSS and ASS data. The Committee noted that based on total liver cancer deaths among the A-bomb survivors, the relative mortality risk was estimated to be 1.26 per Gy, but that if metastases of other cancers to the liver were excluded, the relative risk (1.12) was not statistically significant. Similarly, the Committee noted the ASS cohort did not show a statistically significant increase from an estimated X-ray dose of 163 ± 126 cGy, and no evidence was found following radiotherapy for uterine cancer. The Committee noted that the thorotrast data indicate a liver cancer risk of about

300 per million person-cGy, and that the risk from internal beta emitters "may be about 10 times lower."

Bone

The Committee reviewed the current data on radiogenic bone cancer from both high and low-LET exposures of human populations, and reached the following general conclusions:

- Although human data on bone cancer induced by X- or gamma rays indicates that large doses can cause bone cancer, the dosimetry is too uncertain to permit precise estimates of risk;
- The LSS data provide no evidence of excess bone cancer resulting from gamma doses as high as 400 cGy;
- The most definitive dose-response relationship for radiogenic bone cancer comes from exposure to internally deposited alpha emitters (Ra-224, 226) for which the excess lifetime risk was found to be about 0.02 per person-Gy;
- Studies with beagle dogs indicate a non-linear, concave upward dose-response relationship from internally deposited Sr-90; the risk from Ra-226 in beagles was estimated to be up to 25 times greater per unit dose than Sr-90.

No useful estimates of human risk from low LET irradiation were provided.

Brain and Nervous System

The Committee concluded that although both human and animal data indicate that radiation exposure can cause nervous system tumors, and that the brain is relatively sensitive to the induction of radiation-induced brain cancer, there are not adequate data to permit quantification of risk.

Ovary

Based on its review of current animal and human data, the Committee found that ovarian cancer can result from radiation exposure. The strongest evidence for human cancer was concluded to be from the A-bomb survivors where the relative risk was estimated by RERF to be 2.33 per Gy (DS86 dosimetry). Other studies showed either no statistically significant increase, or were equivocal.

Uterus

The Committee reviewed the recent A-bomb data and other studies (ASS and women treated for cervical cancer) and concluded there are inadequate data to quantify the risk of uterine cancer.

Testis

The Committee reviewed the current data and concluded that there is insufficient information to conclude that testicular cancer is induced by radiation. That is consistent with the most recent RERF conclusions on the A-bomb survivors.

Prostate

The Committee found that although there has been no indication of an increased risk of prostate cancer associated with radiation exposure based on studies up to the early 1980's, there are now data suggesting a weak association. Reports reviewed included the most recent RERF results, the ASS cohort, British nuclear workers, U.S. radiologists, and Swedish patients receiving I-131 for hyperthyroidism. The Committee concluded that though there may be a weak association between prostate cancer and radiation, the sensitivity is comparatively low and not subject to quantification.

Urinary Tract

The Committee reviewed the recent RERF DS86 subcohort of the LSS cohort, the recent ASS cohort data, and data from patients treated with radiation therapy for benign conditions and for cervical cancer. It concluded that although not all studies to date show a statistically significant increased risk, the overall epidemiological evidence shows that radiation can cause cancer of the bladder and, to a lesser degree, cancer of the kidneys. The Committee apparently endorsed the most recent RERF data (DS86) which indicate a relative risk of 2.3 (90% confidence interval, 1.5- 2.4) for urinary tract cancer per Gy, and an absolute risk of 0.7 deaths per million person-yr-cGy.

Parathyroid Glands

The Committee noted that the parathyroid glands were included in the 1980 BEIR III report as tissues susceptible to radiogenic neoplasia, based on observations of a relatively small number of patients given radiation therapy for cervical tubercular adenitis. Typically, latent periods were in excess of 30 years. Although the epidemiological and experimental animal data all indicate that hyperparathyroidism (HPT), parathyroid hyperplasia and adenoma, and less often, parathyroid carcinoma result from radiation doses in the range of 100 to 500 cGy, the Committee did not quantify the risks.

Nasal Cavity and Sinuses

The Committee concluded that there are no human data on dose-response of cancers of the nasal cavity or cranial sinuses from low-LET irradiation.

Skin

The BEIR V Committee reviewed the risk of skin carcinomas resulting from irradiation, and concluded that even though skin cancer carries a low risk of mortality and is grossly underreported, the results of several studies suggest that the risk of skin cancer resulting from irradiation is higher than has previously been reported. Results of studies of infants X- irradiated for enlarged thymus glands (average dose 330 cGy) indicate an average relative risk of 1.5 per Gy, and an average absolute risk of about 0.66 per million person-yr-cGy (<400 cGy) to 0.32 per million person-yr-cGy (>400 cGy).

The Committee noted that the tinea capitis cohort has now been followed for about 30 years since irradiation, and that the latency period is about 20 years. The absorbed dose to the scalp averaged about 450 cGy (range of 3.3-6.0 Gy), while the dose to margin of the scalp averaged 240 cGy and the dose to the face and neck averaged about 10 to 50 cGy. Tumors were observed more commonly in the

lower exposed areas at the margins of the scalp, suggesting that the ultraviolet radiation from the sun may have enhanced the X-ray effects. The lifetime incidence risks of skin cancer were estimated to be about 3.3 cases/cm² per million person-cSv for skin areas exposed to both X-rays and UV, and 0.071 cases/cm² per million person-cSv for skin exposed to X-rays alone (primarily areas of skin covered by hair).² The dose response appeared linear.

Lymphoma and Multiple Myeloma

The Committee noted that only multiple myeloma and non-Hodgkin's lymphoma have been observed to increase as the result of irradiation. Multiple myeloma is expressed as an uncontrolled proliferation of tumor cells (from B lymphocytes) in the bone marrow, while non-Hodgkin's lymphoma is a proliferation of malignant B lymphocytes in the lymph nodes.

Numerous studies have reported increases in multiple myeloma more than any other human lymphoma, and have resulted from both internal and external irradiation. Excess risk has been observed in both U.S. (Hanford) and U.K. nuclear workers, and among ankylosing spondylitics, and has been confirmed and quantified among A-bomb survivors. The A-bomb survivor data indicates that the relative risk per Gy is about 3.29 (90% confidence interval, 1.67-6.31), corresponding to an absolute excess risk of 0.26 per million person-yr-cGy. The latent period is about 20 years for those exposed between 20 and 59 years of age.

With regard to non-Hodgkin's lymphoma, the data were found to be inconsistent by the Committee. While the ASS cohort and women treated for benign gynecological disorders both demonstrate an increased risk, the data from the A-bomb survivors have not yet shown any increased risk. Similar inconsistencies have been observed between studies of early radiologists (showed an increase) and more recent radiologist cohorts (no increase) or pioneer Chinese X-ray workers.

Pharynx, Hypopharynx, and Larynx

The Committee concluded that these tissues are relatively insensitive to the carcinogenic effects of radiation, since cancers in these tissues have only been observed after very high doses of therapeutic radiation (3,000-6,000 cGy) and no excess has been observed in other populations, such as the A-bomb survivors.

Salivary Glands

The Committee found that excess cancers of the salivary glands have been observed in patients given radiotherapy for diseases of the head and neck, in the A-bomb survivors, and in persons exposed to diagnostic X-rays.

For those exposed to radiotherapy (X-rays and I-131 treatments), the results are consistent and yield an average relative risk of 6.9 ($\pm$ 5.5) per Gy (excluding the first 5 years post-irradiation), and an average excess risk of 0.26 cancers per million person-yr-cGy following doses in the range of 40 to over 100 cGy (X-rays).

² corrected values for BEIR V, p. 326 by personal communication from Dr. William H. Ellett, Study Director (BEIR V), National Research Council, May 4, 1990.

In the A-bomb survivors, although there has been no measurable increase in mortality, the incidence has shown a dose-dependent increase that is somewhat smaller than seen among patients receiving radiotherapy.

The Committee concluded that the average excess relative risk of salivary cancer (incidence) is about 550% per Gy, and the average absolute excess risk is about 0.26 cases per million person-yr-cGy. (Note: the relative risk number appears inconsistent with the value cited earlier; it may be possible that the correct value is $690\% \pm 550\%$).

Pancreas

The Committee concluded that there are no study cohorts which have exhibited a reliable, quantified risk of pancreatic cancer to date, suggesting that this organ is relatively insensitive to radiation carcinogenesis.

In Utero Exposure

The BEIR V Committee reviewed the current information related to the potential lifetime risk of cancer and effects on growth and development associated with radiation exposure during pregnancy.

The Committee noted that increased sensitivity to cancer from *in utero* irradiation has not been observed in experimental animals, and the subject has been hotly debated since the Oxford Survey of Childhood Cancers was published over 30 years ago. Since that study, the data have been reanalyzed to include more recent data as these populations continue to age. The Committee found reasonable agreement between the most recent results among the Japanese A-bomb survivors and a 1988 reevaluation of the Oxford data (214 cancer deaths per million person-cGy), and noted an increased risk among 1,429,400 children in the U.S. exposed to diagnostic X-rays between 1940 and 1960. The Committee reported 1988 RERF results which indicated a relative risk (based on DS86 doses) of 3.77 per Gy (90% confidence interval, 1.14 - 13.48) and an absolute risk of 6.57 per million person-yr-cGy (90% confidence interval, 0.47 - 14.49).

The effects of *in utero* irradiation on growth and development has been studied for years in experimental animals, and have included gross structural deformities, growth retardation, embryo lethality, sterility, and central nervous system (CNS) abnormalities. Particularly sensitive is the developing nervous system during early organogenesis. Human studies have also documented similar effects. Although detailed DS86 dose estimates for children exposed *in utero* among the Japanese A-bomb survivors are not yet available, estimates of doses to mothers within 1,600 meters (Hiroshima) or 2,000 meters (Nagasaki) of the bomb epicenters indicate that severe mental retardation clearly resulted from doses above about 20 to 40 cGy during the periods between 8 weeks and 25 weeks of development (the risks were not significantly different from zero for those exposed before 8 weeks or after 26 weeks). The period 8 to 15 weeks was the most sensitive period, with severe retardation in nearly 30% of those exposed to 50 - 99 cGy (average dose of 64 cGy), and nearly 80% in those exposed to over 100 cGy (average dose of 138 cGy). The Committee found the observed risk to be consistent with a linear, nonthreshold dose-response.

Cataracts of the Ocular Lens

In the case of cataracts of the lens of the eye, the Committee concluded that there is a threshold on the order of 60 cGy. Although detectable injury of the eye can occur from doses as low as about 100 cGy, depending on the LET of the radiation the threshold for cataract formation under conditions of highly fractionated or protracted exposure is probably at least 800 cSv. Thus, cataracts are not believed to be a significant risk from normal occupational or public radiation exposures.

Life Shortening

Life shortening has been observed in laboratory animals for many years, and was originally attributed to nonspecific accelerated aging. However, more careful studies showed that the major cause of life shortening was accelerated onset of cancer. The Committee noted that researchers such as Luckey have attributed beneficial or hormetic effects from exposure to low-level, whole-body irradiation, but questioned the relevance of such conclusions to the risks of radiation-induced mutagenic and carcinogenic effects in human populations.

Sterility and Fertility

The Committee also reviewed recent data on the risks of fertility and sterility of adults and concluded that the estimated threshold for induction of permanent sterility in males is about 350 cSv and about 250 to 600 cSv for females, when received in a single exposure.

Genetic Effects of Radiation

Based on their review of the relevant data from humans, other mammals, and mice the BEIR V Committee concluded that the values in Table A.7 provides the best current estimates of the risks of exposure to ionizing radiation. The Committee noted the absence of any statistically demonstrable increase in risk among children of exposed parents in Japan, but nevertheless felt the three probable mutations observed in 667,404 tests for rare electrophoretic variants of 28 proteins versus three probable mutations in 466,881 tests among the comparison groups (one or both parents were beyond 2,500 meters from the bomb epicenters) represents a meaningful indication of effects. This appears more wishful thinking than a true demonstration of effect, but the Committee used these data to estimate the lower 95% confidence limits of doubling dose, and found an indication that genetic risks in human populations may be lower than among experimental animals. Having done that, however, the Committee was unwilling to accept the results and instead went back to the experimental animal data which have always been the basis for estimating human risks from exposure to ionizing radiation. The current estimate of 100 cSv as the doubling dose was based on studies of mice. This represents a conservative estimate of human risk; based on the same limited Japanese data, an estimate of about 468 cSv can be derived. However, given the numerous uncertainties related to estimates of risk related to high-dose, high dose-rate studies to low-dose, low dose-rate risks, the risk estimator is probably as reasonable, and prudent as can be derived at the present time.

Table A.7. Estimated Genetic Effects of 1 cSv per Generation per Million Liveborn Offspring^a

<u>Disorder</u>	<u>Current Incidence</u>	<u>Additional Cases First Generation</u>	<u>(1 Rem/Generation) Equilibrium</u>
Autosomal Dominant			
Severe Effect	2,500	5-20	25
Mild Effect	7,500	1-15	75
X-Linked	400	<1	<5
Recessive	2,500	<1	Slow Increase
Chromosomal			
Unbalanced			
Translocations	600	<5	Little Increase
Trisomies	3,800	<1	<1
Congenital Abnormalities	20,000-30,000	10	10-100
Other Disorders of Complex Etiology			
Heart Disease ^b	600,000	Not Estimated	Not Estimated
Cancer	300,000	"	"
Selected Others	300,000	"	"
TOTALS:	1,000,000	≥16 to ≤53	≥110 to ≤206

^aAssumes a doubling dose of 100 cSv, which is about the lower 95% confidence interval for very limited human data, and is consistent with the estimated doubling dose for mice.

^bNot to imply that any form of heart disease is caused by radiation among the population; any effects result from spontaneous mutations from radiation (and other causes) expressed in future generations as a genetic component of susceptibility (analogous to environmental risk factors that contribute to the environmental component of susceptibility). The magnitude of the genetic component to heart disease and other disorders with complex etiology is unknown (The Committee declined to attempt to quantify the risk, which could be small or as large or larger than all other traits combined). Much more research is needed to sort out the genetic and environmental causes.

A.2.3 United Nations Scientific Committee on the Effects of Atomic Radiation

In 1988 the UNSCEAR Committee report (UNSCEAR, 1988) to the United Nations was published to update its previous reports on the effects of ionizing radiation on man. Unfortunately, most of the textual information presented regarding the Hiroshima and Nagasaki survivors is simply a restatement of older data, although there is a small section generally covering the risks based on the new DS86 dose data. Useful information on the Hiroshima and Nagasaki risks is presented in the tables to this report, especially those by the UNSCEAR Committee. UNSCEAR also presents a summary of studies on occupationally exposed adults which concludes there are as yet no reliable estimates of cancer risk from those sources of exposure.

The UNSCEAR summary and conclusions indicates that estimated lifetime cancer mortality risks from uniform whole-body irradiation at high doses (50 to 600 cGy) and dose rates of low-LET radiation are in the range of 400 to 1,100 per million person-cGy. For low doses and dose-rates UNSCEAR recommended dose reduction factors of between 2 and 10, implying a range of about 40 to 550 cancer deaths per million person-cGy. The UNSCEAR estimates for whole-body irradiation based primarily on the Japanese data (and the new DS86 dose estimates) are reproduced in Table A.8 (Table 71 of the UNSCEAR report).

Table A.8. Summary of Projected Lifetime Risks for 1000 Persons (500 of each sex)* Exposed to 100 cGy of low-LET Radiation at High Dose Rate

<u>Population</u>	<u>Risk Projection Model</u>	<u>Excess Cancer Mortality</u>	<u>Years of Life Shortening</u>
Total Population	Absolute	40 - 50	950 - 1200
	Relative	70 - 110	950 - 1400
Working Population (Ages 25 - 64)	Absolute	40 - 60	880 - 1330
	Relative	70 - 80	820 - 970
Adult Population (Age over 25)	Absolute	50	840
	Relative	60	620

*(Based on the Japanese Population).

In addition, the UNSCEAR Committee also developed a projection of the excess cancer deaths by site of malignancy based on the current Japanese data (Table 69). A summary table of those projections (both relative and absolute risks) is reproduced in Table A.9. Values in parentheses are the calculated 90% confidence intervals (CI).

Following is a summary of the UNSCEAR review for somatic and genetic risks of partial body or organ irradiation potentially relevant to this study.

Table A.9. Projection of Excess Lifetime Mortality for Specific Cancers for 1,000 Persons Exposed to 100 cGy of Organ Absorbed Dose of Low-LET Radiation at High Dose Rate

<u>Malignancy</u>	<u>Multiplicative (Relative) Risk Projection Model</u>	<u>Additive (Absolute) Risk Projection Model</u>
Leukemia	9.7 (7.1-13)	9.3 (7.7-11)
All Cancers Except Leukemia	61.0 (48-75)	36.0 (28-44)
Bladder	3.9 (1.6-7.3)	2.3 (1.1-4.0)
Breast ^a	6.0 (2.8-10.5)	4.3 (2.2-6.9)
Colon	7.9 (3.6-13.4)	2.9 (1.4-4.6)
Lung	15.1 (8.4-23.0)	5.9 (3.4-8.8)
Multiple Myeloma	2.2 (0.6-5.1)	0.9 (0.3-1.7)
Ovary ^a	3.1 (0.9-6.8)	2.6 (0.8-4.8)
Esophagus	3.4 (0.8-7.2)	1.6 (0.3-3.1)
Stomach	12.6 (6.6-19.9)	8.6 (4.5-13.1)
Remainder	11.4 ^b <u>11.8^c</u>	10.3 ^b <u>6.6^c</u>
Total	70.7 ^d 71.2 ^e	45.3 ^d 41.6 ^e

^aThese values have to be divided by 2 to calculate the total and other organ risks.

^bThis value is derived by subtracting the sum of the risks at the sites specified from the risks for all cancers except leukemia.

^cThis value is derived by fitting a linear relative risk model to the basic cancer data at the specific sites listed (Coefficient 0.19 excess relative risk per Gy (100 rads) and 1.87 per 10⁴ PYGy (million person-yr-cGy)).

^dRed bone marrow plus all other cancers.

^eRed bone marrow plus all other individual sites including the remainder.

A.2.3.1 Cancer Risks

Annex F of the 1988 report provides the update of UNSCEAR's prior estimates of radiation induced cancer risks. UNSCEAR acknowledged the major source of concern which is the cause of this report; the uncertainty associated with extrapolation of the effects observed "at high doses and dose rates to the low levels of exposure that may be expected in environmental and occupational settings." UNSCEAR defines high doses to be 200 to 1,000 cGy, and high dose rates to be greater than 5 cSv per minute; low doses are less than 20 cGy, and low dose rates are less than 0.005 cSv per minute.

A.2.3.1.1 *In Utero* and Childhood Exposures

The first group to be addressed by UNSCEAR was children, beginning with a consideration of tissue sensitivity *in utero*. Reviews of emerging cancer data from children of women exposed during pregnancy and children exposed as a result of the nuclear explosions in Japan have produced equivocal data at best.

The so-called Oxford survey (Stewart and Kneale, 1970), reported that *in utero* exposure during the first trimester of pregnancy resulted in almost an order of magnitude increase in cancer risk. Subsequent reports, still claim an increased risk of cancer. Unfortunately, neither animal studies nor studies in Japan, the U.S., or elsewhere have confirmed that hypothesis statistically. While UNSCEAR acknowledged that the BEIR III (1980) report provided a cumulative relative risk of 5.0 for the first trimester and 1.47 for later exposures (total *in utero* risk of 53 cancer deaths per million person-year-cSv), it took a much more circumspect wait and see attitude, provided no risk estimates, but concluded it would be prudent to assume an effect (especially for leukemia induction) until there is adequate data to resolve the question. In the interim, UNSCEAR recommended a relative risk value of 1.58 for leukemia as in its 1986 report, which is described as significant at the 0.05 level. (For a summary of the available data, see UNSCEAR, 1988, Table 14.)

For children treated post-natally with radiation for a primary cancer, there was agreement that all second cancers in the area of irradiation were due to the radiation therapy. However, there was no quantitative risk guidance provided by UNSCEAR.

There is also a substantial body of information presented on children treated with radiation for a number of other disorders, such as retinoblastoma, Wilm's tumor, Ewing's sarcoma, and Hodgkin's disease. The usefulness of such observations for cancer risk estimates for normal populations, however, is debatable since many of these diseases are related to genetic defects (for example, Wilm's tumor and retinoblastoma) or other preexisting malignancies (e.g., Ewing's sarcoma and Hodgkin's disease). As a result, our review has focused on risks to otherwise normal children.

Cancers induced from irradiation of the head (ringworm of the scalp) and neck (enlarged thymus, hemangiomas, enlarged tonsils, etc) include primarily leukemias, and tumors of the thyroid (malignant and benign). Table 20 indicates increased risk ranges from less than 1 percent to about 8 percent for leukemias and thyroid cancer following absorbed doses in the range of 100 to 1,000 cGy, and excess risks of about 1 per million person-year-cGy for leukemia, and about 3.5 per million person-year-cGy for thyroid cancer. Similar results were found for children exposed to nuclear fallout in the Marshall Islands.

According to UNSCEAR, The best dose-response information for the risk of thyroid cancer risk is believed to come from Japanese bomb survivors (even though the most recent data do not indicate an increased risk of thyroid cancer) and from thymus irradiated children in the U.S. The latter children experienced a relative risk of thyroid cancer of 49.1 (29 years of follow-up), and an absolute risk of 5.25 per million person-year-cGy for females and 2.05 for males. In Japan, exposed persons under 19 years of age exhibited about 2.1 per million person-year-cGy, with a sex ratio of about 2.9 (all ages) However, it should be noted that the dose estimates were based on the older T65D dosimetry, are undoubtedly incorrect, and should be considered approximations only. UNSCEAR also notes that nearly all these cancers are relatively benign papillary type, and the mortality risk after 25 years of follow-up is only about 7%. Further, children appear to be at higher risk from exposure than the general population, and additional data are expected as the population under study continues to age. In summary, UNSCEAR concluded that the studies of childhood induction of cancer provide convincing evidence of increased risks, but do not provide useful risk coefficients for lifetime risk projections.

A.2.3.1.2 Cancer Risks among Patients Treated with Radiation

Use of cancer data from people being treated for malignant diseases has two major problems associated with radiation risk estimation: first, it is known that some people have genetic predispositions to cancer (e.g., colorectal and breast cancer), and second, many cancer patients are treated with chemotherapy agents (especially alkylating agents) that greatly increase the risk of subsequent cancer (and there is always the uncertainty as to whether subsequent cancers represent new tumors, or are secondary as the result of metastasis). While many of these uncertainties result from medical practices (e.g., lumping leukemias), confounding factors continue to make evaluation of radiogenic cancer risk very uncertain, since selection of a suitable control population is not straightforward, and these populations are probably not representative of the entire population (due to predisposition).

Hodgkin's Patients

For patients treated for Hodgkin's disease, the most notable second cancer is acute non-lymphocytic leukemia (ANL) which is not believed to be associated with radiotherapy, although solid tumors and other leukemias are observed. Interestingly enough, X-ray therapy alone does not seem to be a major risk factor for the leukemias. That is probably due to the fact that most Hodgkin's patients also receive chemotherapy, which has been implicated in onset of ANL after treatment.

A very recent study (Boivin and O'Brien, 1988) of second cancers after radiotherapy alone indicates that the relative risks for solid tumors were as follows: all sites, 2.2; bones and joints, 20; soft tissues, 18.3; non-Hodgkin's lymphomas, 8.1; skin melanomas, 6.7; buccal cavity and pharynx, 4.1; nervous system, 3.6; respiratory system, 2.5; and digestive system, 1.8. There was no detectable increase in second solid tumors in the chemotherapy-only group, possibly due to the shorter follow-up time.

However, UNSCEAR points to other studies where the effect of radiotherapy when combined with chemotherapy appears to be at most, weak relative to the effects of chemotherapy alone. Unfortunately, effective chemotherapy for Hodgkin's disease is relatively recent, so survival time data is still limited.

As a result, there are no dose-response data available at this time. However, the cumulative relative risk of ANL from chemotherapy treatment of Hodgkin's disease alone is already extremely high, running in the hundreds; for other leukemias it is about 10, and for solid tumors, about 3 to 4. Surprisingly, the risk from chemotherapy is greater for people over 40 than for the young (just the reverse of the effects of radiation). A surprising side-effect of chemotherapy was recently observed in female Hodgkin's disease patients; a two- to five-fold increase in the risk of carcinoma of the cervix and anogenital region (presumably from papillomavirus infections in patients with suppressed immune systems). However, it is not yet possible to identify radiation effects separately.

Cervical Cancer Patients

Women treated by external X-ray beam therapy or implantation of intra-cavity radium sources typically receive fractionated pelvic doses on the order 2,000 to 7,000 cSv over a 4 to 8 week period. Over 180,000 women from eight countries with over 1.3 million person-years of observation are included. The statistical relative risks of second cancers following therapy were as follows: rectum, 1.8; bladder, 3.5; other genital cancers, 3.2; lung, 2.3; breast, 0.7; oral cavity, 1.7; and brain, 0.6. It is interesting to note that the relative risks of breast and brain tumors declined to less than normal rates although these organs received much lower doses (about 10 cSv) due to distance and attenuation in the body. (It is unknown whether these reductions in risk are due to the effects of competing risks (i.e., the patients died of other causes before they developed these cancers), or other complications). Statistically significant cancer risks and their 90% confidence intervals for women followed for more than 10 years are shown in Table A.10.

While the dose to the red bone marrow is estimated to have ranged between 300 and 1,500 cGy, and hundreds of cases of leukemia would have been anticipated, less than 100 were observed, suggesting that cell killing in bone marrow must have been a major factor. Other studies, however, did show relative risks of 2.5 (not statistically significant - average red marrow dose of about 780 cGy) and 2.3 (95% confidence interval: 0.2-24.4). Further studies with the international data mentioned earlier have now confirmed that the leukemia deficit is due to cell killing, and indicate the maximum relative risk of leukemia is about 5 (higher doses lead to lower risks per unit of dose due to cell killing).

A prospective study in Japan indicated a relative leukemia risk of 11.2 from a mean red marrow dose of 120 cGy (excess risk of 0.45 per million person-year-cGy). A subsequent Japanese study of women irradiated for uterine cancer indicated that the relative risks were 3.9 for leukemia and 2.9 for cancer of the rectum.

Ovarian Cancer Patients

Additional information is available for women treated for ovarian cancer in the U.S. The overall relative risk of cancer was 1.4; 1.5 for irradiated patients and 1.1 for non-irradiated patients. Sites included the endometrium (highest rate during first two years after treatment), colon, bladder, breast, and hematopoietic system. Relative risks were fairly constant from 2 to 9+ years, which is too early to draw meaningful conclusions about projection effects, and dose information was not available.

Table A.10. Statistically Significant Cancer Risks for Women 10 or More Years Post-Treatment and the 90% Confidence Intervals

<u>Second Cancers</u>	<u>Relative Risk at 100 cGy</u>	<u>Absolute Risk Excess Cases per 10⁶ PYRad</u>
Colon	1.00 (0.00-1.02)	0.01 (-0.03-0.18)
Cecum	1.02 (0.99-1.09)	-
Rectum	1.02 (1.00-1.04)	0.06 (0.00-0.16)
Female Genitals (total)	1.01 (1.00-1.02)	0.05 (-0.01-0.17)
Ovary	1.01 (0.98-1.14)	0.05 (-0.03-0.60)
Vagina	1.01 (0.98-1.14)	-
Other Genital	0.98 (0.95-1.07)	0.01 (-0.02-0.03)
Bladder	1.07 (1.02-1.17)	0.12 (0.01-0.30)
Connective Tissue	0.95 (0.89-1.13)	-0.01 (-0.02-0.03)
Stomach	1.69 (1.01-3.25)	3.16 (0.05-10.40)
Pancreas	1.00 (0.72-1.62)	0.00 (-0.65-1.43)
Kidney	1.71 (1.03-3.24)	1.10 (0.06-3.50)
Breast	1.03 (0.13-2.29)	0.54 (-14.6-21.7)
Thyroid	13.3 (0.00-77.0)	6.87 (-2.04-39.2)
Leukemia ^a		
CLL	1.00 (0.90-1.43)	0.00 (0.00-0.17)
AL and CML	1.14 (1.00-1.45)	0.10 (0.00-0.31)

^aCLL=chronic lymphocytic leukemia; AL=acute leukemia; CML=chronic myeloid leukemia.

In one of the U.S. groups treated for ovarian cancer, leukemia risks among irradiated patients was not significantly increased, but for those undergoing chemotherapy only, the relative risk was 9.3 and statistically significant (95% confidence interval; CI: 5.2-15.3). Comparisons between patients receiving radiation and chemotherapy and those receiving only chemotherapy indicated no significant difference between the two groups (combined therapy, RR = 120, 95% CI: 44-261; chemotherapy alone, RR = 100, 95% CI: 37-218).

However, another large U.S. study provided conflicting results, with no explanation yet in sight. Relative risks of leukemia in patients believed to have been treated with radiotherapy only, were estimated at 10, while for chemotherapy alone, the relative risk was 9.5, both statistically significant at the 1% level. What is even more perplexing is that when ANL alone was considered, the relative risks among the two groups increased to 21.2 and 22.2, respectively. These findings are totally inconsistent with all other study results.

Breast Cancer Patients

Second cancer data from women treated with radiotherapy only is generally consistent, although here too, there are contradictions. A 1983 study reported no significant evidence of a radiogenic risk to the second breast, but did find a relative risk of 3.2 for all second primary breast cancers relative to the overall public. For breast cancer patients treated only by surgery, the relative risk was 1.2 to 1.4 after five years of follow-up. A 1985 study of the same population reported a relative risk of 2.0 for a second tumor (95% CI: 1.9-2.1) for women treated with radiotherapy, versus a relative risk of 1.5 (95% CI: 1.5-1.6) for women treated without radiation. However, the statistical differences vanished for those surviving 20 years or more after irradiation (RR = 1.7). Data on other cancers (including leukemia) are presented but with little confidence that they are meaningful.

A large 1985 British study found no evidence of increased risk of cancer in the second breast as a result of radiation treatment of the first breast cancer. A 1984 U.S. study also found no significant relative risk for all leukemias, but reported a significant relative risk of ANL following radiation treatment (RR = 3.7, 95% CI: 1.6-7.2), and surgery or chemotherapy (RR = 6.7, 95% CI: 4.5-32.3). The study suggested that unreported chemotherapy may have occurred following radiotherapy, though there may be some radiogenic effect. No dose-response data were available.

Radiation Treatment of Patients for Immune Suppression

In recent years, total-body immune suppression has been induced by irradiation of patients with leukemia or Hodgkin's disease, or for patients preparing for bone marrow or an organ transplant. The results are still uncertain due to the limited follow-up time and small numbers of survivors for most cases. However, there have been some cautious estimates of radiogenic cancer risk for bone-marrow transplants following radiation killing of leukemic blood cells (about 700 to 1,200 cGy to the total body). For 2 to 5 years follow-up, there is currently about a 2% risk of second malignancies, but even those results may be confounded by the fact that some of the patients also received chemotherapy before or accompanying the irradiation.

There is some support for such estimates from another study of patients treated for non-Hodgkin's lymphoma (NHL) where there is an observed relative risk of 105 (95% CL: 48-199) following 2,203 person-years of observation (average follow-up of 4.3 years). For total nodal to total body irradiation, the relative risk of acute non-lymphocytic leukemia (ANL) was 28.0, and 7.0, respectively; both were statistically significant. This study also found suggestions of a correlation between cumulative marrow radiation dose and risk of ANL independent of chemotherapy effects, but the numbers were too small to derive a dose-response relationship. This apparent contradiction between results from treatment of non-Hodgkin's lymphoma (typically 10 cGy per exposure with up to a few hundred cGy total) versus those for Hodgkin's disease may be the consequence of the larger doses given Hodgkin's patients (typically 200 cGy per day for a total dose of thousands of cGy) which killed cells that might have produced acute non-lymphocytic leukemia.

An additional risk of immune suppression is susceptibility to human papilloma virus leading to an increased risk of cervical and related cancers.

However, the result is not a direct radiogenic cancer risk and is not dose dependent.

Leukemogenesis Following Radiotherapy

In a large U.S. study reported in 1984 following radiotherapy for all types of cancer, there was only a significant increase in relative leukemia risk associated with chemotherapy. The relative risk of leukemia following radiotherapy without chemotherapy was inconclusive. Relative risks significant at the 5% level or better following radiation therapy alone were only observed for endometrial and ovarian cancer, 2.1 and 10.0 respectively. However, the relative risk for all sites was 1.4 at the 1% level. See UNSCEAR, Table 29 for details.

A 1986 study of radiation-induced leukemias in four states used chronic lymphocytic leukemia (CLL) as a control for all other leukemias, since it has not been linked to radiation exposure. The relative risk of CLL after radiotherapy was 0.7 (not significantly different from 1.0), while the relative risk of all other leukemias was statistically significant: 1.6 for all irradiated sites and 2.4 for trunk sites alone. However, no dose-response data were available.

Leukemia and Multiple Myeloma

Risk data from non-cancer related radiation treatments are probably the most reliable source of radiation risk data. In the case of leukemia, the bulk of the information comes from Japanese bomb survivors and ankylosing spondylitis patients. Unfortunately, UNSCEAR was unable to factor the DS86 dose estimates into their report; as a result, the conclusions must be taken with caution, and this review will be more limited than would be the case if the new Japanese DS86 data had been used. However, in both studies, the first evidence of increased risk of leukemia occurred within 5 years after exposure, and has remained elevated for at least 40 years. Yet if bomb survivors under 15 years of age are excluded to permit comparisons with the spondylitis patients, the relative risk to bomb survivors (13.5) is much greater than for the spondylitics (3.37). Part of the difference could be due to cell killing among the spondylitics, or to the more limited amount of marrow exposed. Neither study showed an increase in the relative risk of chronic lymphocytic leukemia. The relative risk and excess cases (UNSCEAR, Table 30) for leukemia among the ankylosing spondylitics and Japanese survivors (using the incorrect T65D estimates) are shown in Table A.11 (given the recent DS86 dosimetry, the risk among Japanese survivors would be revised upwards about a factor of two, making the disparity even larger).

It should be noted that chronic granulocytic leukemia peaks within 5 to 10 years post-exposure among the Japanese survivors regardless of age at exposure, then declines to near background within about 20 years post-exposure. On the other hand, for all adults, the peak periods of expression occur later in life and tend to be spread out over longer periods of time.

Acute and chronic granulocytic leukemias display very similar patterns of expression among those survivors under 15 years of age at the time of exposure. (See UNSCEAR, 1988, Figure V).

Table A.11. Relative Risk and Excess (Absolute) Cases for Leukemia

<u>Population</u>	<u>Relative Risk</u>	<u>Excess Cases per 10⁶ PY</u>
Ankylosing Spondylitis	4.79 (3.4-6.6)	1.96 (1.24-2.88)
Japanese Survivors	9.38 (7.0-12.6) ^a	3.95 (3.04-4.86)

^aIndividuals exposed to >100 cGy vs. those exposed to <10 cGy (T65D).

In the case of multiple myeloma, the Japanese data exhibit a 15 to 25 year latency period with an extended period of risk, but with no differences in relative risk between the sexes. Excess risk (based on the faulty T65 dose estimates) was 0.48 per million PYcGy. Relative risk ranged from about 1.6 to 2.3 for low-LET exposures, and about 4.3 for primarily high-LET exposures. See UNSCEAR, Table 32 for details.

Cancer Risks in Non-epithelial Tissue

Data on risk for actively dividing mesenchymal tissue are strongest, and for cancers of the tissues of the periosteum of the bone (e.g., osteosarcoma), radiation is the only well-documented risk factor. The internally deposited, high-LET alpha-emitters Ra-226,228 are among the best known causes of bone cancer. Radiogenic osteosarcomas tend to occur in the same locations of the skeleton in which spontaneous osteosarcomas occur, especially near the epiphyses of rapidly growing long bones (e.g., the knee joint). Note: this could also be taken as evidence that the cancers are from osteogenic progenitors lining the internal bone surfaces (i.e., endosteal).

Osteosarcomas were also observed among German patients given Ra-224 for treatment of ankylosing spondylitis, bone tuberculosis and other diseases. The appearance of these sarcomas follows the same time course as radiogenic leukemias among Japanese bomb survivors. The speculation is that the much shorter biological half-life of Ra-224 makes its exposure more like a brief exposure than for Ra-226. The mean dose received ranged from about 205 cGy in adults to about 1,100 cGy in children, delivered over a period of 6 and 11 months, respectively. It was estimated that the absolute risk coefficient for Ra-224 was about 27 bone sarcomas per million person-cGy.

Epithelial Tissues

Epithelial tissues are often characterized by populations of stem cells that actively divide throughout their life-times to produce the differentiated cells that provide the structure and functional basis of some organ systems. The most common radiogenic cancers originating from epithelial cells are those of the breast, lung, and thyroid. Less common are those of the skin, digestive organs, liver, pancreas, and uro-genital system.

(1) Breast Cancer

A large number of women have received chest X-ray treatments or fluoroscopy for a variety of medical conditions. These include tuberculosis, post-partum mastitis, and artificial pneumothorax. In addition, breast irradiation was experienced by large populations in Japan from the nuclear explosions which ended World War II. Although UNSCEAR has not yet updated earlier estimates of risk to account for the new DS86 dose estimates, there is no doubt that breast tissue is very susceptible to radiogenic cancer, especially for those exposed as children. Relative risk among Japanese survivors declines from about 9 for children aged 0 - 4, to around 5 for ages 5 - 10, then declines gradually to about 2 - 3 for ages 20 and older. An average absolute risk was estimated to be 3 - 4 per million PYcGy (based on faulty dose estimates), and is expected to increase when the new dosimetry is applied. SAIC notes that when that happens the risk coefficients should more closely agree with risk coefficients from U.S. experience with medical exposures, as discussed below.

U.S. mastitis patients exhibit risk coefficients about twice that of the Japanese survivors (6 to 8.5 per million PYcGy), with a relative risk of 3.2 (90% CL: 2.3-4.3). The linear dose-response and multiplicative (relative) risk projection was a better fit of the data.

The Canadian fluoroscopy data also indicate a relative risk on the order of 2 - 3 for doses of 100 to 400 cGy, with higher values for those exposed at a younger age. However, the Canadian dose-response data is best fit by a pure quadratic model, though a linear-quadratic model fits almost as well.

(2) Lung Cancer

Most of the exposures related to medical exposures to the chest and breast, also resulted in lung irradiation. At the present time, UNSCEAR finds no difference between lung cancer risks between Japanese men and women after smoking effects are accounted for. In addition, there is a large body of information from exposure of underground miners exposed to radon progeny.

For brief low-LET exposures (primarily Japanese bomb survivors and ankylosing spondylitis patients), relative risks appear to be about 1.2 - 2.0. Miners exposed to high-LET radon progeny over long periods of time show mixed results, depending on the country where they were exposed. Risks are usually presented per working-level-month (WLM) of exposure (170 hours exposure to 1 working level). Czechoslovakian data indicates lifetime risks of about 450 per million WLM, while Ontario, Canada data indicates about 170 per million WLM. U.S. data were generally ignored.

Overall, UNSCEAR suggests that a range of 150 - 450 lung cancer deaths per million WLM is reasonable. If one assumes "usual conditions in mines" a reference conversion factor of 0.6 cGy to the bronchi per WLM can be derived. That implies a lifetime risk of about 90 - 270 lung cancer deaths per million person-cGy (an assumed RBE of 20 would give a range of 4.5 to 13.5 lung cancer deaths per million person-cSv).

UNSCEAR reported the relative risk model developed by BEIR IV (1988) which also accounts for age specific risk:

$$r(a) = r_0(a)[1 + 0.025g(a)(W_1 + 0.5W_2)]$$

where $r(a)$ is the lung cancer mortality rate at age a ; $r_0(a)$ is the baseline lung cancer mortality rate in the 1980-1984 U.S. population; $g(a)$ is a coefficient equal to 1.2 for ages under 55, 1.0 for ages 55-64, and 0.4 for ages 65 and older; W_1 is the cumulative radiation exposure in WLM from 5 to 15 years before age a ; and W_2 is the cumulative exposure 15 years or more before age a . BEIR IV concluded that 1 WLM per year would increase the number of lung cancer deaths by a factor of 1.5 for current patterns of smoking.

(3) Thyroid Cancer

The data on thyroid cancer risk among children has already been discussed, and more details are shown in Table 20 of the UNSCEAR report. Adult thyroid exposures as a result of radioiodine therapy for hyperthyroidism has yet to show any signs of excess cancer, especially the most dangerous anaplastic form. Data from fallout of radioiodine from U.S. weapons test in Nevada also do not indicate any excess thyroid cancer among the exposed population, although doses have been as high as 150 cGy following exposures from multiple weapons tests (primarily in children). Estimates of excess risk range from about 1 to 4 per million PYcGy.

Swedish studies of patients given radioiodine for diagnostic evaluations have also failed to identify any excess cancer risk for typical doses of 50 to 150 cGy after 17 years of follow-up. An expansion of this study later revealed some increased risk for patients receiving the higher doses for detection of suspected tumors. Further, it was estimated that the effectiveness of the prolonged exposure was about a third to a fourth of that for a single acute dose.

In Japan, bomb survivors have shown a relative risk of about 4, with an excess risk of about 0.92 (males) and 2.40 (females) per million PYcGy. The approximate 3:1 ratio observed is about the same as among the non-exposed population.

The National Council on Radiation Protection and Measurements derived the following absolute risk equation:

$$\text{Risk} = R \times F \times S \times A \times Y \times L$$

where R is the absolute risk of 2.5 per million PYcGy for both sexes in ethnically similar populations of children exposed to X-rays after a minimum latent period of 5 years; F is the dose effectiveness factor taken as 1 for external X or gamma-irradiation and I-132, 133, and 135, or 0.33 for I-131 and 125. S is a sex correction factor equal 1.33 for females and 0.667 for males; A is a age-susceptibility correction factor equal 1 for ages under 18, and 0.5 for ages over 18; Y is the average number of years of post-exposure risk in the group being evaluated; and L is lethality, taken to be 0.1 (based on papillary tumor induction). Calculated results using this equation for internal exposures to I-125, 131 and external exposures to X or gamma radiation are presented in Table 39 of the UNSCEAR report. Lifetime excess mortality risk ranges from 0.274 to 1.45 for males (before age 18 and after) and 0.68 to 3.15 for females (before age 18 and after) per 10,000 individuals exposed to between 6 and 150 cGy.

(4) Other Epithelial Cancers

Cancers in other epithelial tissues are still being studied, but dose-response information is generally poor or nonexistent. Some excess risk values are presented for urogenital, liver and digestive system, but there still

substantial uncertainty in the estimates, and the further review of that information was judged to be unproductive.

A.2.3.2 Genetic Risks

Annex E of the report UNSCEAR report discusses current uncertainties and perspective on genetic risks relative to radiation exposure and other causes. Prevalence of Mendelian diseases in the general population is composed of about 50 entities, the individual birth frequencies of which range from about 10^{-4} to about 10^{-5} , with some allowance for as yet undiscovered diseases. UNSCEAR estimates that diseases associated with chromosomal defects (particularly deletions and duplications) have been detected on every human chromosome. Microdeletions have been associated with malformed children, especially those predisposed to several types of cancer. However, prevalence and contribution to disease has not yet been quantified. One exception has been "fragile sites" on X-chromosomes which is related to mental retardation. This has been estimated to be about 4×10^{-4} , making it second only in prevalence to Down's syndrome as a disease resulting from of a chromosomal abnormality. However, there is no information yet regarding the susceptibility of the condition to induction by radiation

Previous estimates of congenital anomalies and diseases of complex etiology (0.043 prevalence) and other multifactorial diseases (0.047 prevalence) were based on a 1974 British Columbia survey which was limited to observed effects from birth to age 21. UNSCEAR notes that more recent studies of the Hungarian population, indicates that the prevalence of congenital anomalies and multifactorial diseases may be much higher, on the order of 0.06 and 0.6, respectively, when individuals through age 70 are included. Unfortunately, almost nothing is known regarding the mechanisms or the induction of such diseases from radiation exposure.

Another current area of interest are movable genetic elements in the role of spontaneous mutations and chromosomal aberrations. Recently, short interspersed elements (SINEs) and long interspersed elements (LINEs), ranging from less than 500 base pairs, and a few hundred base pairs up to about 7,000 pairs, respectively, have been observed in mammalian cells as repetitive DNA sequences. However, there is currently no information linking these transposable elements to spontaneous mutations in man. Current studies of DNA repair in mammalian cells indicate that DNA repair takes place preferentially on active (transcribing) genes. As a result, it appears that damage to the silent regions of the human genome may be more likely to result in mutations. However, UNSCEAR believes it is still not possible to define the role of radiation exposure in germ cell mutagenesis.

UNSCEAR noted the recent studies of human spermatozoa irradiated *in vitro*, which indicates that chromosomal aberrations increase linearly with X-ray dose, rising from the spontaneous rate of 14% to 19% at 25 cGy, 29% at 50 cGy, 43% at 100 cGy, and 68% at 200 cGy. However, the risks associated with such aberrations has not yet been quantified.

Genetic studies of the Hiroshima and Nagasaki populations continue, but have not yet revealed any direct evidence of mutations associated with the exposures. UNSCEAR noted that new studies of genetic effects in offspring of cancer patients exposed to radiation and chemical agents, and among those populations exposed to

radiation from the Chernobyl accident have been initiated and may produce useful information in the future.

Current UNSCEAR genetic risk estimates from exposure to low level, low LET radiation are essentially the same as provided in the 1982 and 1986 UNSCEAR reports, and are summarized in Table A.12.

Table A.12. Genetic Health Effects per Million-Person-cSv

Genetic Effect	First Generation Genetic Effects			Equilibrium (All Generations)
	Males	Females	Average	
Dominant Mutations	10 - 20	0 - 9	15	100
Recessive Mutations	0	0	0	15
Unbalanced Reciprocal Translocations	1 - 15	0 - 5	2.4	4
TOTALS:	11 - 35	0 - 14	18	119

A.2.4 National Radiological Protection Board (NRPB) Findings

In December 1988, the NRPB published it's report "Health Effects Models from the 1988 UNSCEAR Report" (NRPB, 1988). Much of the information used in deriving the models came from UNSCEAR, 1988 but the risk coefficients for latent effects selected were specific for UK populations, even though the models were based primarily on the Japanese A-Bomb survivor data (Ibid, pp.14-15). Subsequent to the RERF efforts in developing the dose estimates for the I85D dose estimates, the new dosimetry system was developed, entitled DS86 (Roesch, 1987). The DS86 dose estimates were made for approximately 76,000 survivors (out of the total of approximately 91,000 in the Life Span Study group; a substantial increase from the RERF I85D estimates).

The NRPB addressed numerous concerns associated with applying the Japanese data to other populations such, as the UK. These involved special considerations of the differences in spontaneous cancer rates in the female breast (much lower than in the west), and stomach (much higher than in the west). The NRPB concluded that the relative risk model was more appropriate for use with most cancers (solid tumors), while the absolute risk model was most appropriate for leukemia. In the case of breast cancer, the NRPB used the relative risk coefficients derived from North American women irradiated for medical reasons (e.g., mastitis) rather than the LSS data from the Japanese survivors.

NRPB found that for leukemia, the absolute risk is high for those irradiated before age 10, then falls, and rises again for older ages at exposure. Age-specific relative risks given by Shimizu et al. (1988) were used for all cancers

other than leukemia (NRPB, 1988, pp.22-23), leading to higher projected risks for solid tumors than would be projected from either an age-averaged relative risk model or from an absolute risk model. NRPB dealt with the extrapolation of risk coefficients based on high doses and/or dose rates (HDR) to low doses and low dose rates (LDR) by using a dose-rate effectiveness factor (DREF). For dose rates below 10 cGy/day and low doses (less than 1 cGy), NRPB concluded a DREF of 2 was applicable for the female breast, while a DREF of 3 applied to all other tissues. These values are generally consistent with the recommendations of UNSCEAR (1988) which suggested a range from 2 to 10 as appropriate for most cancers.

NRPB used two methods of projecting risk over time for the UK population (baseline cancer rates of England and Wales, and the 1985 UK life table); the first was based on a projection of risks over a full lifetime, and the second was based on the actual risk observed to the present time (i.e., 40 years post exposure). It is the belief of NRPB that these two methods bound the probable lifetime risks. The NRPB estimates of the risks from leukemia and other cancers are summarized in Table A.13, and compared with the earlier ICRP (1977) estimates.

A.2.4.1 Leukemia

The HDR values for leukemia in Table A.13 were based on the age and sex-specific absolute risk coefficients for the Japanese survivors, assuming an RBE of 10 for neutrons, although due to the much smaller neutron doses calculated, the choice of RBE has little effect on the calculated risk. The expression period was assumed to be 2 to 40 years after exposure. A DREF of 3 was assumed appropriate for LDR, low LET exposures.

A.2.4.2 Breast

The HDR values for breast cancer were based on age-specific relative risk coefficients derived from studies of patients in New York given X-ray therapy for acute postpartum mastitis, and from tuberculosis patients in Massachusetts who received large fluoroscopy doses. The results were averaged over a population of males (approximately zero risk) and females. A DREF of 2 was assumed appropriate for LDR, low LET exposures.

A.2.4.3 Thyroid

The HDR values for thyroid cancer were based on age-specific absolute risk coefficients recommended by the NCRP (1985) which in turn were derived from a review of medically exposed children in the U.S. (e.g., tinea capitis and thymus irradiation). NRPB assumed that the expression period commenced 5 years after irradiation and continued until the end of life (or 40 years post exposure). The ratio of mortality to incidence was assumed to be 10:1. A DREF of 3 was assumed appropriate for LDR, low LET exposures.

A.2.4.4 Bone

The LDR values for bone cancer were based on an absolute risk model, with coefficients derived from radium dial painters in the U.S. The model assumes an RBE of 20 for alpha radiation, with an expression period beginning 2 years after exposure commenced and continuing until 40 years post exposure. The DREF for low-LET exposure was taken to be three at low dose rates.

Table A.13. Estimated Excess Cancer Mortality Risks (per 10,000 cGy) for the UK Population from Exposure to Low LET Radiation at High (HDR) and Low (LDR) Dose Rates

Cancer Type	Lifetime Mortality Risk		Risk Observed to Date ^g		ICRP-1977 (Adults)
	HDR ^h	LDR ^h	HDR	LDR	
Leukemia ^a	0.84	0.28	0.84	0.28	0.2
Breast ^b	1.1	0.55	0.42	0.21	0.25
Lung ^a	3.5	1.2	1.15	0.38	0.2
Thyroid ^c	0.075	0.025	0.055	0.02	0.05
Bone ^d	0.15	0.05	0.15	0.05	0.05
Liver ^e	0.45	0.15	0.23	0.08	0.1
LLI/colon ^a	1.1	0.37	0.38	0.13	0.1
Stomach ^a	0.73	0.24	0.14	0.05	0.1
Remainder ^{a,f}	5.0	1.63	0.565	0.20	0.2
TOTAL:	12.9	4.5	3.93	1.4	1.25

^aLower large intestine/colon; based on Japanese A-Bomb survivors.

^bBased on studies of western women.

^cFrom NCRP Report No. 80 (1985); also Appendix A, NRPB (1988).

^dBased on radium dial painters; Appendix B, NRPB (1988).

^eBased on Thorotrast cases (BEIR, 1988); Appendix B, NRPB (1988).

^fDifference; Appendix B, NRPB (1988) for skin.

^gBased on period of follow-up of Japanese to date (40 years).

^hHDR = high dose rate (> 10 cSv/day); LDR = low dose rate (< 10 cSv/day); DREF=2 for breast, 3 for others.

A.2.4.5 Liver

The LDR values for liver cancer were based on the BEIR IV (1988) review of studies of Thorotrast patients (mainly in Germany). An absolute risk model was used to project risk beginning 20 years after start of exposure up to end of life (or 40 years). The DREF for low-LET exposure was assumed by NRPB to be 3 for LDR.

A.2.4.6 Lung, Colon, Stomach and Remaining Tissues

The HDR values for lung, colon, stomach and remaining tissues were based on age and sex-specific relative risk coefficients for the Japanese survivors,

assuming a neutron RBE of 10. The expression period was assumed to begin 10 years after exposure and continue until end of life or until 40 years post exposure. One exception was colon cancer where the risk coefficients for exposure before age 20 were replaced with values from the 20 to 29 year group due to the lack of data for the former. Remaining tissues include cancers of the esophagus, ovary, bladder, rectum, kidney, and multiple myeloma. A DREF of 3 was used to estimate risk coefficients for LDR, low LET exposures.

A.2.4.7 Skin

This risk coefficient came from ICRP (1977) recommended values, assuming 1% of all skin cancers are fatal. A DREF of three was assumed for low dose and LDR, low LET radiation exposures. The LDR value for cancer mortality was assumed to be 1×10^{-6} per cGy for low-LET exposures.

Additional information summarizing the results of the UNSCEAR (1988) relative and absolute risk estimates and assumed fatality rates for radiation-induced cancer are summarized in Tables A.14 and A.15.

A.2.4.8 In Utero Exposure

A special category of the human population at risk of cancer from radiation exposure are those exposed *in utero*. The principal source of information on such exposures was the Oxford Survey of Childhood Cancers (OSCC), which is still on-going. While the study has produced controversial results during the last 15 years, NRPB feels the recent data has now produced a reasonable estimate of cancer risks associated with *in utero* exposures. A recent revelation is that the risk of exposure in the first trimester, long thought to be the most sensitive phase, is now believed to be no greater than the other trimesters (NRPB, 1988, p.40). The relative risk based on the OSCC is thought to be about 1.4 (i.e., the risk of childhood cancer following *in utero* X-irradiation is about 40% higher than normal). NRPB notes that the risk estimates from Japanese survivors are extremely uncertain (95% confidence interval is 1.14-13.5 for relative risk), but bound the OSCC estimate (Ibid, p.42).

Citing UNSCEAR (1988) and BEIR III (1980), NRPB concluded that the risk of childhood cancer incidence is about 600 per million person-cGy (250 cases of leukemia and 350 solid tumors). Since about half of these childhood cancers are fatal, NRPB concluded that the mortality risk coefficients for leukemia and solid tumors are about 125 and 175 (300 total) per million person-cGy for LDR, low dose, low LET exposures.

A.2.4.9 Genetic Effects

In addition to the future risks of radiation-induced cancer, there is also a risk associated with future hereditary (genetic) effects. Recognizing the absence of measurable genetic risk in the 30,000 offspring of Japanese survivors, NRPB has followed the time-honored method of relying on animal data for estimating the risks of various types of genetic abnormalities (Ibid., p.32). Although there have been no measurable genetic effects observed yet in children of Japanese survivors, the studies to date have permitted an estimate of the lower limit of the "doubling dose" (dose necessary to double the spontaneous rate of genetic damage). One study estimated that the doubling dose was 46 cGy for fathers and 125 cGy for mothers (Ibid., p. 32), and another study estimated the

Table A.14. Projected Lifetime Risks of Cancer Mortality per cSv Whole Body Low-LET Exposures at HDR (UNSCEAR, 1988)

<u>Exposed Population</u>	<u>Projection Model ($\times 10^{-4}$)</u>	
	<u>Relative Risk</u>	<u>Absolute Risk</u>
Population of all ages ^a	7 - 11	4 - 5
Workers (ages 25-64)	7 - 8	4 - 6

^aThe ranges reflect the differences between age-specific and age-averaged risk coefficients.

Table A.15. Assumed Fatality Rates for Radiation-Induced Cancers (ICRP, 1985)

<u>Cancer</u>	<u>% Fatalities</u>
Leukemia	90
Breast	50
Lung	95
Thyroid	10
Bone	80
Liver	95
ILI/colon	75
Stomach	95
Skin	1
Remainder (Including Stomach)	75

Ref: Table 7, NRPB (1988)

doubling dose to be approximately 140 to 180 cSv (Ibid, p.33). BEIR III provided a range of 50 to 250 cGy (BEIR III, 1980, p.110).

Genetic effects may occur from changes in the base sequences of DNA in single genes leading to a mutation that is not lethal to the cell (i.e., only a non-lethal mutation in a germ cell is heritable). Mutations may be dominant, recessive, and X-linked. Dominant mutations are always expressed in the first generation (if not lethal) regardless of the other allele. However, recessive mutations are not expressed if they have a matching normal allele. As a result, recessive mutations may not be observed for many generations when the specific mutations become common enough to be present on matching alleles from both

parents. An exception are mutations of genes on the X chromosome (X-linked) which may be expressed in males whether dominant or recessive, since the matching allele is nearly always missing from the Y chromosome.

Changes in normal chromosome number in man (46 pairs; 23 from each parent) is nearly always fatal to a developing fetus, or will lead to severe physical and/or mental defects in newborns (e.g., Downs Syndrome).

Other types of hereditary disorders are referred to by NRPB as "multifactorial" diseases. Relatively common chronic diseases may have a genetic component but environmental influences play a role in disease expression. UNSCEAR (1988) and NRPB estimates of hereditary disorders at equilibrium are summarized in Table A.16 along with the estimates taken from BEIR III (1980) for comparison. For comparison, the ICRP (1977) estimated the total risk of genetic effects was 2×10^{-4} per cSv.

Table A.16. Incidence of Genetic Disease at Equilibrium from Parental Exposure per million cSv per Generation per million Liveborn

<u>Disease Classification</u>	<u>BEIR III (1980)</u>	<u>UNSCEAR (1988)</u>	<u>Natural Incidence per million live births</u>
Chromosomal Animals	Slight Increase	4	3,800
Dominant and X-linked	40-200	100	10,000
Recessive	Very Slow Increase	15	2,500
Multifactorial	-	-	60,000-600,000
Irregularly Inherited	20-900	-	-
Total:	60-1,100 (GM = 260)	120	76,000-620,000 ^a

^aBEIR III gives an estimate of about 110,000 (BEIR, 1980, p.85).

NRPB recommends a risk coefficient of 80 per million person-cGy for low dose and low dose rate exposure to low LET radiation, and 240 per million person-cGy for HDR and high doses (NRPB, 1988, p.34). This assumes a genetically significant dose delivered during the first 30 years of life, with effects expressed over all future generations.

A.2.5 Other Studies

A.2.5.1 Breast Cancer Among Women Treated for Acute Postpartum Mastitis (APM)

An ongoing study of women given X-ray therapy for APM during the 1940's and 1950's was recently updated (Shore *et al.*, 1986). The study included 601 women and 1,239 controls, and covers a post-exposure period of up to 45 years, with an average of 29 years. The study concluded that the relative risk model provided the best fit to the observed data (e.g., the age and temporal patterns of cancer occurrence, and the average latency did not vary by dose), and the response was approximately linear with dose (the doses ranged from 69 to 1,400 cGy, although only 35 breasts received over 800 cGy). Interestingly enough, the risk per cGy for the various exposure groups appeared to be independent of the dose fractionation, which ranged from 10 to 230 cGy per treatment. Nevertheless, the study produced breast cancer absolute risk coefficients of 7 to 9 per million person-year-cGy, consistent with the BEIR III estimate of 6 per million person-year-cGy.

A downward curvature of the dose-response curve at high doses was attributed to cell killing.

A.2.5.2 Review of the Risk of Cancer from *In Utero* Exposure

Following a review of 2,802 individuals who were exposed *in utero*, and utilizing the most recent reassessment of doses (DS86), Yoshimoto *et al.* (1988) concluded that for all individuals whose dose exceeded 1 cGy, the risk of cancer through 1984 was 40.9% higher. The relative risks are summarized in Table A.17.

Table A.17. Risk of Cancer from *In Utero* Exposure

<u>Dose Range</u>	<u>Excess Cancers</u>	<u>RR</u>
1 to 29 cGy	7 cancers (1.31 excess) out of 682 individuals	1.24
30 to 59 cGy	3 cancers (1.13 excess) out of 129 individuals	2.18
Over 60 cGy	3 cancers (2.88 excess) out of 109 individuals	4.78

The 95% confidence intervals are so large that the lower bound for the most exposed group overlaps the upper bound of the two lower exposed groups.

In order to see if cancer risks for *in utero* exposed individuals are higher than for children exposed postnatally, Yoshimoto *et al.* (1989) compared the observed risks with children exposed at 0 to 9 years of age. No significant differences were found for radiogenic leukemia, although the number of leukemias is very small (2 cases), and subject to large uncertainty. Further, although this study found no difference in sensitivity with gestational period, two cases are not a sound basis from which to draw any solid inferences (see NRPB study above, which does draw such inferences from the ongoing Oxford (OSCC) study).

The relative radiosensitivity of fetal tissues to damage and potential cancer induction has also been reviewed (Brent et al., 1987). That study concluded that the estimate of leukemia risk from Stewart et al. (1970) is not the actual radiation risk, since it includes the spontaneous risk as well. When corrected for the spontaneous rate, Brent et al. (1987) concluded that the leukemia risk for *in utero* exposure is on the order of 1/6,000 per cGy for the 10 years following exposure.

A 1989 review of the Oxford Survey of Childhood Cancers (OSCC) by Gilman et al. () updated earlier estimates by Stewart et al. (1970). As before, dosimetry data is non-existent, so it was assumed the total dose was proportional to the number of X-ray films taken. The authors concluded that the relative risk of leukemia following *in utero* exposure during the first trimester of pregnancy was 2.73 times that from exposure in the following two trimesters. This study has a few substantial problems; first, over 90% of the total number of cases studied occurred in the 3rd trimester (1,317 out of a total of 1,443 cases); second, only 51 cases out of the total of 1,443 cases resulted from 1st trimester exposure (i.e., the statistical power is weak, compared to the 3rd trimester data); third, the 3rd trimester showed no increased risk as a result of *in utero* exposure; and 4th, this data is generally inconsistent with the data from Japanese bomb survivors or other studies. Another source of concern is the fact that most of the cases during the 1st trimester were the result of reasons other than related to pregnancy (e.g., chiefly pyelograms and other contrast media examinations), and resulted in greater numbers of films than obstetric examinations. However, there is no information presented regarding differences in dose per film, etc. The ultimate value of these studies remains to be determined, but it appears that the most reliable conclusion is that there is no increased risk from exposure during the 3rd trimester.

A.2.5.3 Risk of Thyroid Cancer from Thymus Irradiation

Shore et al. (1985) reassessed the risk of thyroid cancer in a group of 2,652 persons who received X-ray treatment for purported enlarged thymuses in infancy (erroneous diagnoses for most infants). Average time since exposure was about 29 years. While thyroid doses ranged from 5 to over 1,000 cGy, 62% of the doses were less than 50 cGy (mean dose 120 cGy). To date, 30 thyroid cancers and 59 benign thyroid adenomas have been detected as compared to 1 thyroid cancer and 8 adenomas among a control group of 4,823 siblings. For thyroid cancer, the absolute risk model appeared to best fit the data, although there was excess risk for both cancers and adenomas for at least 40 years post-exposure. For both cancers and adenomas, the absolute risk per cGy was 2 to 3 times greater in females than in males, and there was no indication that risk was affected by dose fractionation.

An earlier study suggested that ethnicity may have been important, since the risks appeared to be higher among Jewish patients. However, that was found not to be the case in this study, and was attributed to the fact that the Jewish patients in general received larger doses than the other patients due to differences in treatments (Shore et al. (1985), p.1179).

The absolute risk coefficient derived from this study was 3.46×10^{-6} per person-year-cGy (PYcGy) for cancer and 5.7×10^{-6} per PYcGy for adenomas (Ibid, p.1180). The relative risk coefficients were 0.58% per cGy for cancers and 0.44% per cGy for adenomas. Both models were adequately described by a linear dose response model (there were small or insignificant quadratic components in the RR

model), and there was no indication of a multiplicative risk with respect to age (i.e., it was an additive, absolute risk).

A.2.5.4 Cancer Risk Associated with Irradiation for Tinea Capitis (Ringworm of the Scalp)

Ron et al. (1988) reassessed the risk of cancer among 10,834 patients of African or Asian parentage who received 75 to 100 kVp X-rays for Tinea capitis in Israel between 1948 and 1960 compared to 10,834 matched controls and 5,392 siblings. The average follow-up period was 26 years. The organ-specific relative and excess risk coefficients are summarized below for specific neoplasia which account for the major risks observed to date (Table A.18).

Table A.18. Relative and Excess Risk Coefficients from Irradiation of the Scalp (Tinea Capitis)^a

Neoplasia	RR (95% CI) vs. Controls	ER (95% CI) vs. Controls	RR (95% CI) vs. Siblings	ER (95% CI) vs. Siblings
Head & Neck	2.9 (1.2-7.2)	4.6 (0.5-15.4)	6.0 (1.5-39.5)	6.1(1.3-96.6)
Leukemia	2.8 (1.0-8.7)	3.2 (0.1-13.7)	2.0 (0.6-7.6)	2.9(-1.1-18.9)
Other Cancers	0.9 (0.5-1.9)	-0.3 (-2.8-5.1)	0.7 (0.3-3.0)	-2.2(-5.0-14.4)

^aRR is relative risk, CI is confidence interval, and ER is risk per 10⁻⁵ PY

For non-leukemias, essentially all of the excess risk was associated with tumors of the head and neck. 57% of the leukemias, 70% of the head and neck tumors, and 60% of the brain and CNS tumors were attributed to childhood irradiation. Absolute risk estimates per million persons per year per cGy were 0.81 (0.01-2.8) for leukemia (average bone marrow dose of 30 cGy), 0.15 (0-0.7) for CNS neoplasms (assuming an average brain dose of 140 cGy). Cancer risk was highest for those children irradiated between the ages of 5 and 10. The highest period of risk was the five years following exposure, and was due primarily to leukemia (Ibid, p.719). Solid tumors began to appear about 15 years post-irradiation, and remained elevated until 25 years of observation (Ibid, p.720).

The results of this study must be viewed with some caution, due to the potential loss of data due to deaths of study participants during the Six Day (1967) and Yom Kippur (1973) wars in Israel. Military service accounted for 21% of total deaths, while cancer related to therapy accounted for only about 7% (Ibid, p.720). The presumption must be made that both the controls and irradiated subjects were at the same competing risk from military service. Since many of the subjects are now reaching the age of increasing spontaneous cancer risk, additional follow-up studies will be required before the risk is accurately known.

A.2.5.5 Cancer Risks from Radiation Treatment of Ankylosing Spondylitis

While the UNSCEAR and BEIR committees both recently reviewed the data still accumulating from exposure of ankylosing spondylitis patients to X-rays, there are some recent reports that were not addressed in those reviews. Of particular interest are two studies in the U.K. which attempt to determine whether the relative or absolute risk models best fit the data.

Muirhead and Darby (1987) reviewed the modeling of the relative and absolute risk models in which families of models were examined using data from the Ankylosing Spondylitis Series (ASS) and the Life Span Study (LSS) sample for Hiroshima (Nagasaki was avoided due to the uncertainties in dose). Two generalized risk models were proposed. The relative risk model:

$$\lambda_i(1) = \lambda_i(0)\{1 + \exp(x_i\beta)\}$$

or the absolute risk model:

$$\lambda_i(1) = \lambda_i(0) + \exp(x_i\beta),$$

where the "hazard rate" in stratum i of the high-dose group, λ_i , may be related to corresponding stratum in the low-dose group, $\lambda_i(0)$, and β is a vector of regression coefficients (estimated from the data), and x_i is a scalar. These two models were then embedded in a parametric family given by:

$$\lambda_i(1) = [\lambda_i(0) + \{1 + \exp(x_i\beta)\}^\gamma - 1]^{1/\gamma}$$

The absolute risk model results from $\gamma = 1$ (since the difference between the risks of high and low dose groups is constant), while the relative risk model arises as γ approaches 0 (the ratio of the risks is constant).

The authors concluded that the ASS data for most cancers is best fitted with a relative risk model, even for leukemia. Thus, the authors found their modeling results consistent with BEIR III except for leukemia which is quite different from interpretations of the LSS data.

In 1988, Muirhead and Darby published a follow-on analysis of the data they developed in the prior study of the relative and absolute risks of all cancers (excluding leukemia and colon cancer; leukemia was examined in their 1987 paper, and "Colon cancer was examined separately since it is associated with the occurrence of spondylitis"). Variables which could be related to radiogenic cancer included sex, age at exposure, age at observation, and time since exposure. Data for the five year period following exposure were excluded because "tumors appearing during this time may have been caused the symptoms that were incorrectly ascribed to spondylitis."

A major criticism of this paper is that there was no attempt to relate variations in dose with response. Instead, the authors chose to fix the dose to the mean estimated dose. The authors concluded that the mean doses were in excess of 100 cSv for most organs, and the mean total body dose was 207 cSv. Given the fact that the over 14,000 patients received similar treatment (although the authors noted that the doses were non-uniformly delivered), the variation in dose may not be significant. However, the authors noted that a report describing the estimated organ-specific doses to the spondylitics will be published soon.

The best fit for the relative risk model (RR) included an exponential expression containing terms relating to age at exposure, and time since exposure (linear and quadratic components). The model predicts a decreasing relative risk with increasing age at exposure for the ages studied (15 to 64). In one analysis, male spondylitics divided into two age cohorts: 15 to 24 or 35 to 44 and relative risks in terms of excess deaths per million per year were compared. Both cohorts showed an initially increasing trend with time since exposure, followed by a sharp drop in risk 20 years after exposure. These results are quite different than observed among the Japanese bomb survivors or other studies where the absolute risk for all cancers except leukemia continue to increase with time; the authors were unable to explain the differences at this time.

For the absolute risk model (AR), the best fit included both linear and quadratic terms for age at observation, and time since exposure. The authors concluded that inclusion of terms for age at observation reflected the need to take into account variations in the baseline cancer rate when modeling absolute risk. The authors conclude such a need does not arise for modeling RR, presumably because the relative risk data inherently include baseline data.

The authors then used their best-fit RR and AR models to project lifetime cancer risks (excluding leukemia) for a hypothetical male population, aged 15 to 64 at time of exposure to a 10 cSv low-LET exposure, assuming a 10 year latent period (same as BEIR III) and expression until the end of life. The results are summarized in Table A.19.

Table A.19. Relative and Absolute Risks from Ankylosing Spondylitis Data

Age at Exposure	No. of deaths/10,000		Years of life lost/10,000	
	RR	AR	RR	AR
15-24	1.7	1.6	61	52
25-34	4.4	5.6	110	142
35-44	10.1	10.3	180	196
45-54	14.9	10.1	180	132
55-64	13.5	4.9	106	43
15-64	8.2	6.2	123	112

Interestingly enough, the RR and AR models are in reasonable agreement for all age cohorts except the 55-64 group, and overall produce very similar projections. A second concern raised by the authors is that there are no useful data for spondylitics that were under 15 years of age at time of exposure. Therefore, follow-up of other populations, such as the Japanese bomb survivors, that include that age cohort will be important in predicting lifetime risks from those exposed as children.

A.2.5.6 Mortality of Hanford Workers: 1945-1981

In January 1989, Gilbert et al. published their latest findings regarding the long-term cancer risk among Hanford workers from occupational exposure. With the exception of a continuing correlation for multiple myeloma (RR of 55% per

cSv), no statistically significant positive correlation was seen between radiation and mortality from all cancers combined, or from leukemia. The worker population still exhibits a strong "healthy worker" effect, with death rates substantially below those of the general U.S. population. The major impact of the study is that it allows an estimate of the upper bound. The RR of multiple myeloma is based on only three cases with relatively high exposure, and is not supported by the Japanese bomb survivor data (RR of 0.51% per cSv based on erroneously higher T65D dose estimates). The authors concluded that the Hanford data supports an upper bound estimate of risks no more than about 20 times the linear-quadratic estimate preferred by the BEIR III committee, or about 7 times the linear estimate. The Hanford-based upper 90% confidence limit for absolute risk of leukemia was about 3 deaths per million PY compared with values of 0.8 assumed in ICRP Publication 26 (1977) and 1.3 derived by the BEIR III committee from a linear-quadratic response function.

A.2.5.7 Patients Treated with Radiotherapy for Cancer of the Cervix

In 1988, Boice, et al. published an update on the risks of radiogenic cancer among 150,000 women irradiated at 20 oncology centers and 19 population-based cancer registries in 14 countries following radiotherapy of primary cancer of the cervix. Of the group treated, 4,188 women had second cancers. This group was compared with 6,880 matched controls. The doses to the cervix and nearby tissues were generally quite high (several hundred Gy), so there was much "wasted" radiation and cell killing. [These doses would almost certainly result in underestimates of risk extrapolated to lower doses]. One strong point in this study results from the fact that since radiotherapy has been the treatment of choice for nearly a half century, few of these patients received chemotherapy which typically confounds the radiation dose-response data. Further, survival is relatively good, permitting good statistics on long-term risks, accurate dose reconstruction is possible, and patients treated only by surgery are available for comparisons.

Radiotherapy treatments included intracavity radium implants, X-rays, cobalt-60, van de Graaff generators, and betatrons. Dose reconstruction was performed by emplacing about 350 TLD's in a phantom at specific organ locations in the body for each measurement.

Cancer risks were significantly increased for cancer of the bladder (RR = 4.0), rectum (RR = 1.8), vagina (RR = 2.7), uterine corpus (RR = 1.3), cecum (RR = 1.5), non-Hodgkin's lymphoma (RR = 2.5), stomach (RR = 2.1), leukemia (ALL + CML; RR = 2.0), kidney (RR = 1.2), and possibly bone (RR = 1.3). Radiation was not found to increase the overall risk of cancer of the small intestine, colon, ovary, vulva, connective tissue, breast, Hodgkin's disease, multiple myeloma (actually much less than controls), or chronic lymphocytic leukemia (which has never been linked with radiation exposure).

A.3 SUMMARY

There have been few large epidemiological studies of the risks of radiation neoplasia in human populations which have followed the exposed populations until the end of life. As a result, there are substantial uncertainties in both the shape of the dose-response curves and lifetime risks. The uncertainties relative to low dose, low dose-rate exposures is large because the only populations for which statistically meaningful results have been demonstrated are those which

were exposed to relatively large doses and usually at high dose rates. The size of the population required to provide statistically meaningful results is approximately inversely proportional to the square of the excess cancer. Thus, if 1,000 samples (human or animal) in both the irradiated and control populations were required to provide adequate statistical power at 1 Gy (100 rad), about a billion in each group would be required at 1 mGy (100 mrad; BEIR III Report, 1980, p. 140). Such large population requirements generally prohibit resolution of the risks of radiation at low doses and low dose rates, due to the inordinately large costs and lengths of the studies that would be involved.

Given the uncertainties, dose response models must be regarded with caution. However, the potential dose-response curves shown in Figure 1.2 represent the best possibilities at this time. The most recent human studies indicate that cancer data are best fit with by a linear dose dependence whereas leukemias are best fit by a linear-quadratic model (BEIR V Report, 1990; UNSCEAR Report, 1989; NRPB Report, 1988). The fact that animal and cell studies generally support the use of these dose dependencies in the same dose and dose-rate ranges provides assurance that the models are probably adequate for analyzing human risks.

Similar uncertainties arise from the use of risk projection models, because it is necessary to estimate the future risk of radiogenic cancer among the many human populations being studied, and most members are still living. The most prominent projection models, which have received widespread use, are the *absolute risk model*--in which the excess cancer risks are assumed to be directly proportional to dose, to be independent of the spontaneous rate of cancer, and to add to the latter rate; and the *relative risk model*--in which the excess cancer risks per unit dose are assumed to be proportional to the spontaneous rate of cancer. These models are discussed in Section 1.4, and illustrated graphically in Figure 1.5. As knowledge of radiation risk has accumulated over the past decade, the majority of radiation epidemiologists seem to support the use of the relative risk model for human risk projections (BEIR V Report, 1990; UNSCEAR Report, 1988; NRPB Report, 1988; and reports from the Radiation Effects Research Foundation, e.g., Shimizu et al, 1987, 1988).

A major factor in the recent reassessments of the risk of radiogenic cancer in humans was the reevaluation of the radiation dosimetry for the Hiroshima and Nagasaki A-bomb survivors which was essentially completed in 1987 (Roesch, 1987). These changes are especially important because most of the current estimates of risks of radiogenic cancer in humans depend on the Japanese populations that were exposed. The principal changes resulting from the new dosimetry are as follows:

- In Nagasaki, the gamma-ray kerma (approximately the first collision dose) was about 10 to 30 percent less and the neutron kerma 50 to 65 percent less than previous estimates.
- In Hiroshima, the gamma-ray kerma was about 2 to 3.5 times larger, while the neutron kerma was nearly a factor of 10 lower than previous estimates.
- The gamma-ray transmission factor for Japanese homes was estimated to be only about half that of earlier estimates; neutron transmission factors remained about the same.

- The average organ transmission factor for survivors was estimated to be higher than before compensating in large part for the reduced gamma-ray transmission factors for houses.
- Due largely to the reductions in neutron and gamma-ray doses, the earlier differences in the observed risks between Hiroshima and Nagasaki survivors have now been resolved, with little difference in the risks per unit dose between the survivors in the two cities.

As noted above, the most recent information regarding human risks of cancer resulting from ionizing radiation comes from four major reviews; all rely heavily on the revised dosimetry and recent cancer data from the Japanese survivors. These studies are:

- The Radiation Effects Research Foundation (RERF) in Japan;
- The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR);
- The U.K. National Radiation Protection Board (NRPB); and
- The U.S. National Research Council's Committee on the Biological Effects of Ionizing Radiation (BEIR).

The results of these studies are discussed in some detail and summarized in several tables in Appendix A of this report. The major revisions resulting from the RERF study, as reflected in the remaining three studies, are summarized in Section 1.5.16 and Table 1.9. However, the differences between the risk estimates in these reviews appear to be largely the result of somewhat different modelling assumptions, and whether or not the estimates developed from high-dose, high-dose-rate data were used to estimate risks for low-doses at low-dose-rates.

In the foregoing reviews, only that of the NRPB (1988) elected explicitly to include dose rate effectiveness factors (DREFs). The report of the NRPB (1988) applied a DREF of 2 for breast cancer and 3 for all other cancers when extrapolating risk from high doses and high dose rates to low doses at low dose rates. The lifetime cancer mortality risks of leukemias (absolute risk) and non-leukemic cancers (relative risk) were estimated to be 28 and 420 per million person-cSv, respectively; not greatly different from the estimates in the BEIR III Report (1980) based upon the use of the relative-risk, linear-response model).

The Report of UNSCEAR (1988) recognized that data from both the ankylosing spondylitic patients and from animal studies clearly showed reduced cancer risk when the same dose given at high dose rates was protracted over long periods of time. Although this Committee recognized the need for DREF adjustments in the range of 2 to 10 when extrapolating from the permissible risks for radiation workers to that for the general public, they declined to provide that specific guidance pending further study. The estimates of the UNSCEAR Committee for leukemias and non-leukemic cancers from high dose, high dose-rate exposures (e.g., as might result from serious accidents at nuclear power plants) were about 93 (absolute risk) and 610 (relative risk) per million person-cSv, respectively.

In the BEIR V Report (1990), while relying on somewhat different models than the Committees of the NRPB and the UNSCEAR, similar estimates of risk were

provided, less than a factor of two difference. The BEIR V Committee argued that the use of its linear-quadratic dose response model for leukemia induction, had a "built-in" DREF of about 2.1 for estimates of doses below about 50 cSv (i.e., where single track events predominate). The estimates in the BEIR V Report (1990) of lifetime cancer risk for leukemias and non-leukemic cancers are 95 (relative risk model) and 695 (relative risk model) per million person-cSv, respectively.

Unless the estimates in the BEIR V Report (1990) and in the UNSCEAR Report (1988) are adjusted for dose rate effects, it is likely that their new estimates will have major impacts on the dose limits that are applied to radiation workers and the general public. This possibility follows because the current limits recommended by the International Commission on Radiological Protection (ICRP) are based on risks estimates that are about 4 to 6 times lower than in these two Reports.

A.4 REFERENCES

- BEIR III, "The Effects on Populations of Exposure to Low Levels of Ionizing Radiation: 1980," (National Academy Press, Washington, D. C., 1980), pp. 1-524.
- BEIR IV, "Health Risks of Radon and Other Internally Deposited Alpha-Emitters," (National Academy Press, Washington, D. C., 1988).
- BEIR V, 1990. "Health Effects of Exposure to Low Levels of Ionizing Radiation," (National Academy Press, Washington, D.C., 1990).
- Boice, J. D., Jr., G. Engholm, R. A. Kleinerman, M. Blettner, M. Stovall, H. Lisco, W. C. Moloney, D. F. Austin, A. Bosch, D. L. Cookfair, E. T. Krentz, H. B. Latourette, J. A. Merrill, L. J. Peters, M. D. Schultz, H. Storm, E. Bjorkholm, F. Pettersson, C. M. J. Bell, M. P. Coleman, P. Fraser, F. E. Neal, P. Prior, N. W. Choi, T. G. Hislop, M. Koch, N. Kreiger, D. Robb, D. Robson, D. H. Thomson, H. Lochmuller, D. V. Fournier, R. Frischkorn, K. E. Kjorstad, A. Rimpela, M-H. Pejovic, V. P. Kirn, H. Stankusova, F. Berrino, K. Sigurdsson, G. B. Hutchinson, and B. MacMahon, "Radiation Dose and Second Cancer Risk in Patients Treated for Cancer of the Cervix," Radiation Research 116, 3-55 (1988).
- Boivin, J. F., and K. O'Brien, "Solid Cancer Risk after Treatment of Hodgkin's Disease," Cancer 61, 2541-2546, 1988.
- Brent, R. L., D. A. Beckman, and R. P. Jensh, "Relative Radiosensitivity of Fetal Tissues," Advances in Radiation Biology, Vol. 12, p. 239 (1987).
- ICRP, Recommendations of the International Commission on Radiological Protection, Publication 26, Annals of the ICRP (Pergamon Press, 1977).
- Kaul, D. C., "Revised Dosimetry System for Atomic Bomb Survivors," in Second US-Japan Joint Workshop for Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki, D. J. Thompson, Ed. (Radiation Effects Research Foundation, Hiroshima, Japan, 1983).
- Kopecky, K. J., and D. L. Preston, "The Impact of the Reassessment of A-Bomb Dosimetry: Dose Estimation Methods and Preliminary Cancer Mortality Findings," RERF TR 11-86, 1988.
- Muirhead, C. R., and S. C. Darby, "Modelling the Relative and Absolute Risks of Radiation-Induced Cancers," Journal of the Royal Statistical Society A, 150, Part 2, pp. 83-118, 1987.
- Muirhead, C. R., and S. C. Darby, "Relative and Absolute Risk Models for Cancer Mortality in Ankylosing Spondylitis Patients," Proceedings of the 14th L. H. Gray Conference (Oxford, U.K., September 1988, in publication).
- NCRP, Induction of Thyroid Cancer by Ionizing Radiation, National Council on Radiation Protection and Measurements (NCRP Report 80, Bethesda, MD, 1985).

NRPB, Strather, J.W., C. R. Muirhead, A. A. Edwards, J. D. Harrison, D. C. Lloyd, and N. R. Wood, "Health Effects Models Developed from the 1988 UNSCEAR Report." National Radiation Protection Board Report NRPB-R226, Chilton, Didcot, Oxon OX11 0RQ, U.K., December, 1988.

Preston, D. L., and D. A. Pierce, "The Effect of Changes in Dosimetry on Cancer Mortality Risk Estimates in the Atomic Bomb Survivors," RERF TR 9-87 (Radiation Effects Research Foundation, Hiroshima, Japan, 1987).

Roesch, W. J. (Ed.), Final Report of the US-Japan Reassessment of the Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki (Radiation Effects Research Foundation, Hiroshima, Japan, 1987).

Shimizu, Y., H. Kato, and W. J. Schull, "Cancer Mortality in the Years 1950-1985 Based on the Recently Revised Doses (DS86)," Life Span Study Report 11, Part 2, RERF TR 5-88 (Radiation Effects Research Foundation, Hiroshima, Japan, 1988).

Shimizu, Y., H. Kato, W. J. Schull, D. L. Preston, S. Fujita, and D. A. Pierce, "Comparison of Risk Coefficients for Site-Specific Cancer Mortality Based on the DS86 and T65DR Shielded Kerma and Organ Doses," Life Span Study Report 11, Part 1, RERF TR 12-87 (Radiation Effects Research Foundation, Hiroshima, Japan, 1987).

Shore, R. E., E. Woodard, N. Hildreth, P. Dvoretzky, L. Hemplemann, and B. Pasternack, "Thyroid Tumors Following Thymus Irradiation," Journal of the National Cancer Institute, 74, No. 6, 1177-1184, 1985.

Shore, R. E., N. Hildreth, E. Woodard, P. Dvoretzky, L. Hemplemann, and B. Pasternack, "Breast Cancer Among Women Given X-Ray Therapy for Acute Postpartum Mastitis," Journal of the National Cancer Institute, 77, No. 3, 689-696, 1986.

Stewart, A., and G. W. Kneale, "Radiation Dose Effects in Relation to Obstetric X-Rays and Childhood Cancers," Lancet 1, 1185, 1970.

UNSCEAR, Sources, Effects, and Risks of Ionizing Radiation: 1988 Report to the General Assembly, with annexes (United Nations, New York, 1988).

USNRC, Reactor Safety Study: An Assessment of Accidental Releases in U. S. Commercial Nuclear Power Plants, WASH-1400, (U. S. Nuclear Regulatory Commission, Washington, D. C., 1975).

Yoshimoto, Y., H. Kato, and W. J. Schull, "Risk of Cancer Among Children Exposed In Utero to A-Bomb Radiations, 1950-84," Lancet, 665, 1982.

Appendix B

APPENDIX B

GLOSSARY

Absolute risk is the arithmetic difference between the risk among those exposed and those not exposed, i.e., the excess above normal expectation.

Acentric fragment is a fragment of a chromosome that lacks a centromere and is often lost at cell division.

Adduct usually refers to the addition of a molecule to DNA thus creating structurally and chemically an abnormal sequence.

Alkylating agent is a positively charged (i.e., electron-deficient) compound, or one which may be metabolized to form such a compound. The reactive groups can form covalent linkages with negatively charged chemical groups on biological molecules such as those on the bases of DNA. A monofunctional alkylating agent can form a single adduct, whereas bifunctional alkylating agents can form two adducts leading to inter- or intra-strand DNA-DNA cross-links or to DNA-protein cross-links.

Allele is one of several alternate forms of a gene occupying a given locus on a chromosome.

Alpha particle is a charged particle emitted from the nucleus of an atom having a mass and charge equal in magnitude to a helium nucleus, i.e., two protons and two neutrons.

Amplification refers to the intracellular production of additional copies of a genetic sequence, found as either intrachromosomal or extrachromosomal DNA.

Anaplasia is the histopathological appearance of a tumor which lacks features allowing easy identification with the tissue of origin. Anaplastic tumors are undifferentiated and usually rapidly growing and have a large number of cells in mitosis.

Antibody is a soluble protein produced by plasma cells in response to an antigen and capable of specifically binding to that antigen.

Antigen is an agent that is foreign (i.e., "non-self") to an animal, is recognized by the immune system as foreign, and provokes synthesis of an antibody (immunoglobulin).

Autocrine is a growth factor or hormone which can influence the metabolism of the cell which produces it.

Autoradiography detects radioactively labeled molecules by their effect in creating an image on photographic film.

Autosomes are all of the chromosomes in a cell except for the sex chromosomes. A diploid cell has two copies of each autosome.

Back mutation reverses the effect of a mutation that had altered a gene; thus, it restores wild phenotype.

Bacteriophages are viruses that infect bacteria; often abbreviated *phages*.

Bands are regions of chromosomes that retain a stain upon certain chemical treatments.

Base pair (bp) is a hydrogen-bonded partnership of adenine with thymine, or of cytosine with guanine in a DNA double helix. Other pairs are possible in RNA under some circumstances.

Beta particle is a charged particle emitted from the nucleus of an atom, having a mass and charge equal to those of an electron.

Cancer refers to a group of diseases characterized by the uncontrolled proliferation of cells, usually leading to local growth, invasion, and to metastasis. Synonyms that are used are neoplasm, malignancy, or malignant neoplasm

Carcinogen is a substance which causes cancer. Some chemical carcinogens can act directly, but others require metabolism *in vivo* before becoming effective. Most carcinogens are also mutagens.

Carcinoma is a type of cancer arising from epithelial tissue (i.e., tissue lining internal or external organs, or glandular tissue, etc.). Most human cancers are carcinomas.

c banding is a technique for generating stained regions around centromeres.

cDNA is a DNA copy complementary to mRNA sequences that were transcribed from a given gene or genes. cDNA will therefore hybridize with these genes and, if radiolabelled, will allow their detection in chromosomes ("*in situ* hybridization") or in DNA extracted from cells and separated by electrophoresis (as in "Southern blots") or in RNA (Northern blots).

Cell cycle is the period from one cell division to the next.

Centromere is a constricted region of a chromosome, visible at division, that includes the site of attachment to the mitotic or meiotic spindle.

Chromatids are the copies of a chromosome produced by replication.

Chromatin is the complex of DNA and protein in the nucleus of the interphase cell.

Chromosome is the structural unit containing the genetic material (DNA) within a cell. Normal human cells usually have 46 chromosomes consisting of 22 pairs of autosomes plus the sex chromosomes (XX in females, XY in males). Different chromosomes may be recognized in metaphase cells by their shape and by their banding patterns.

cis-acting locus affects the activity only of DNA sequences on its own molecule of DNA.

Clastogenic is a property of a chemical or physical agent that results in the breakage of chromosomes.

Clone is a family of cells all derived from one parent cell. A clonal marker (e.g., an abnormal chromosome or protein product) may identify all of the cells within a given clone. Most human tumors appear to arise from a single cell and hence are clonal.

Cloned gene is a gene that has been isolated and inserted into a "vector," usually a plasmid or a bacterial virus. The vector containing the gene can be produced in large amounts, thereby providing genetic material suitable for assays and studies of the function of the gene. Cloned genes can be used to produce large quantities of pure protein products of cells (e.g., insulin, interferons).

Coding region is that part of the DNA which actually codes for a protein. The part of the DNA molecule which is initially transcribed into messenger RNA (mRNA) contains both *introns* and *exons*. The introns are regions of mRNA which are spliced out during post-transcriptional processing. The exons are the regions in the mRNA which comprise the processed message; they contain the coding regions and are, therefore, the "expressed" portion of the gene. The final processed mRNA may have untranslated regions containing important regulatory signals.

Coding strand of DNA has the same sequence as mRNA.

Codon is a triplet of nucleotides that codes for an amino acid in a protein or for the termination of synthesis.

Complementation refers to the ability of independent (non-allelic) genes to provide diffusible products that produce a wild phenotype when two mutants are tested in a heterozygote condition.

Deletion is loss of DNA. Deletions range from small, affecting only a single base pair of a gene, or large, resulting in a chromosomal deletion involving many genes.

Dicentric chromosome is the product of fusing two chromosome fragments, each of which has a centromere. It is unstable and may be broken when the two centromeres are pulled to opposite poles in mitosis.

Differentiation is the development by cells of specific characteristics which provide for the normal functioning of tissues.

Diploid set of chromosomes contains two copies of each autosome and two sex chromosomes.

Disjunction describes the movement of members of a chromosome pair to opposite poles during cell division. At mitosis and the second meiotic division, disjunction applies to sister chromatids; at first meiotic division it applies to sister chromatic pairs.

DNA repair is a complex process that may involve many enzymes and may lead to repair of damage in one or both strands. Repair may lead to complete restoration of the DNA (error-free repair) or may result in alteration or deletion of bases (error-prone repair).

Dominant allele determines the phenotype displayed in a heterozygote with another (recessive) allele.

Dose is a general term denoting the quantity of radiation or energy absorbed. For special purposes, it must be appropriately qualified. If unqualified, it usually refers to absorbed dose.

Absorbed dose is the energy imparted to matter by ionizing radiation per unit mass of irradiated material at the place of interest. The unit of absorbed dose is the rad, or the Gray (Gy). 1 rad = 100 ergs/g, or 0.01 J/kg. 1 Gy = 100 rads = 1 J/kg.

Cumulative dose is the total dose resulting from repeated or chronic exposure to radiation.

Dose-response relationships are the mathematical relationships that describe the frequency of a radiation-related event with increasing dose.

Dose fractionation is a method of administering radiation in which two or more doses are applied, each fraction separated by an interval(s).

Dose rate is the absorbed dose delivered per unit time.

Double minute is a small amount of genetic material that is seen in some cells as a paired body resembling a very small chromosome without a centromere. Because they lack a centromere, double minutes distribute themselves randomly at mitosis and are easily lost during cell growth. Double minutes have often been shown to contain amplified genes.

Doubling time is the time taken for an exponentially growing tumor (or cell population) to double its volume (or number of cells).

Dysplasia refers to abnormal growth of cells characterized by cytologic atypia. Some of the atypical changes may resemble those found in neoplastic cells. Neoplasms often arise from dysplastic tissues and, in some cases, dysplasias are considered pre-cancerous.

Electron volt (eV) is a unit of energy equal to the energy gained by an electron in passing through a potential difference of 1 V. Larger multiple units of the electron volt are frequently used: keV for thousand or kilo-electron volts; MeV for million or mega-electron volts. $1 \text{ eV} = 1.6 \times 10^{-12} \text{ erg}$.

Endocrine is hormone production by a gland at one site in the body that is released into the bloodstream to act on tissues that are distant to that site.

Endonucleases cleave bonds within a nucleic acid chain; they may be specific for RNA or for single-stranded or double-stranded DNA.

Enhancer is a DNA sequence which increases the activity of promoter sequences which are initiators of transcription (i.e., production of messenger RNA). Enhancers can be located anywhere in the noncoding regions of a gene.

Enzymes are proteins that catalyze biochemical reactions.

Epigenetic changes influence the phenotype without altering the genotype.

Episome is a circular form of DNA which replicates in cells independent of the chromosomes. Viral DNA may form episomes in cells. Plasmids used for gene cloning grow as episomes in bacteria.

Euchromatin comprises all of the genome in the interphase nucleus except for the heterochromatin from which it is distinguishable by its staining properties.

Exons are the coding regions of a gene that are found in processed RNA, messenger RNA (mRNA).

Exonucleases cleave nucleotides one at a time from the end of a polynucleotide chain; they may be specific for either the 5' or the 3' end of DNA or RNA.

Flow cytometry is a technique in which cells are tagged with a fluorescent dye and then directed in single file through a laser beam. The intensity of fluorescence induced by the laser light is detected and the number of cells exhibiting different levels of fluorescence is recorded. Cells may also be separated according to the intensity of their fluorescence in a process known as fluorescence-activated cell sorting.

Forward mutations inactivate a normal, wild-type gene.

G banding is a technique that generates a striated pattern in metaphase chromosomes that distinguishes the members of a haploid set.

G₁ is the period of the eukaryotic cell cycle between the last mitosis and the start of DNA replication.

G₂ is the period of the eukaryotic cell cycle between the end of DNA replication and the start of the next mitosis.

Gamete is either type of reproductive (germ) cell--sperm or egg--with haploid chromosome content.

Gene is the segment of DNA that is involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

Gene conversion is the alteration of one strand of a heteroduplex DNA, i.e., base-pair unmatched DNA, to make it complementary with the other strand at any position(s) where there were mispaired bases.

Genomic (chromosomal) DNA clones are sequences of a genome carried by a cloning vector.

Genotype is the genetic constitution of an organism.

Glycoprotein is a protein to which various types of sugar molecule have been attached. Glycoproteins form an important component of the cell surface.

Gray (Gy) is the new international unit (S.I. unit) of absorbed dose equal to 1 J/kg. 1 Gy = 100 rad.

Growth factor is a polypeptide produced by cells which stimulates either the same cell or other cells to proliferate. Several types of growth factor have been isolated and some of these may be associated with abnormal regulation of growth in transformed cells. Growth factors interact with cells through specific receptors in the cell membrane.

Haploid set of chromosomes contains one copy of each autosome and one sex chromosome. The sex chromosomes in a male cell are *hemizygous* because there is only one copy of the X and Y chromosomes

Heterochromatin describes regions of the genome that are permanently in a highly condensed condition and normally are not genetically expressed.

Heterogeneity is variability in the properties of cells within an individual tumor. Wide heterogeneity of many properties is found among cancer cells.

Heterozygote is an individual with different alleles at some particular locus.

Homeostasis is the maintenance of a normal physiological state. Homeostasis is often maintained through a balance between regulatory feedback systems employing signals (e.g., hormones) that may have opposite effects.

Homogeneously staining region (HSR) is a region which appears uniform on chromosomes stained to examine their banding pattern. It often represents amplification of genes. HSR's tend to be stably inherited by daughter cells.

Homologues are chromosomes carrying the same genetic loci; a diploid cell has two copies of each homologue, one derived from each parent.

Homozygote is an individual with the same allele at corresponding loci on the homologous chromosome.

Hotspot is a site at which the frequency of mutation (or recombination) is very much increased.

Housekeeping (constitutive) genes are those (theoretically) expressed in all cells because they provide basic functions needed for sustenance of all cell types.

Hybridization is (a) the fusion of two cells to form a single cell, (b) the association to each other of complementary (homologous) sequences of DNA or RNA. Radiolabelled pieces of DNA or RNA can be used as *probes* to identify specific DNA sequences by hybridization. The technique may localize genes to specific chromosomes in a process known as *in situ* hybridization.

Hyperplasia is an increase in the rate of proliferation of non-neoplastic cells that exceeds that of normal cells in that particular tissue. Hyperplastic changes in tissues sometimes precede the formation of a neoplasm.

ICRP, International Commission on Radiological Protection.

ICRU, International Commission on Radiation Units and Measurements.

Immortalization is the process which may allow cells to form a continuous cell line (i.e., proliferate indefinitely) in culture. Normal cells will proliferate for only a limited number of passages in culture and immortalization appears to be a necessary but not a sufficient step in transformation to a neoplastic state.

Immune surveillance is a proposed mechanism whereby the immune response recognizes the development of neoplastic cells at an early stage and inactivates them before they can develop into tumors.

Immunoglobulin is an antibody molecule.

Incidence is a term used in epidemiology to describe the number of new cases of a certain disease that is observed in a specific population in a given interval of time, usually one year.

Incidence rate is the rate of occurrence of a disease within a specified period of time.

Induced mutations result from the application of a mutagen.

Inducer is a small molecule that triggers gene transcription by binding to a regulator protein.

Induction is the process by which a virus, whose genetic material is integrated into the cellular DNA, is caused to be released from the cell as a mature virus.

Initiation is the first stage in the process of carcinogenesis. It involves interaction of the carcinogen with the DNA of the target cells to produce, after DNA replication, a permanent lesion. Subsequent steps include promotion and progression.

Insertions are identified by the presence of an additional stretch of base pairs in DNA.

Integration is the process by which viral DNA, or DNA copies of the RNA of a retrovirus, are incorporated into the chromosomal DNA of a cell.

Interphase is the period between mitotic cell divisions.

Intron is a noncoding region in the internal portion of a gene. These regions are spliced out during processing of the initial mRNA transcript.

Invasion is infiltration by cancer cells into neighboring normal tissues. It is one of the distinguishing features of malignancy.

Ionizing radiation is radiation (e.g., X- or gamma-rays) which is sufficiently energetic that it can cause formation of ions during its passage through matter (tissue).

Karyotype is the array of the chromosomal content of a particular cell displayed by photographing the chromosomes in a metaphase cell, cutting the individual chromosomes out of the photograph and ordering them according to a standard notation.

kb is an abbreviation usually for 1000 base pairs of DNA, or 1000 bases of RNA. **kbp** can be used to specify base pairs in DNA.

Latent period is the period or state of seeming inactivity between the time of exposure of tissue to an injurious agent and a recognizable response. In the induction of neoplasia, it is the interval between the inducing treatment and the detection of the disease.

Lethal locus is any gene in which a lethal mutation can be obtained.

Leukemia refers to a group of neoplastic diseases affecting the various cells of the blood-forming, hematopoietic system. All the cell lineages derived from the progenitor cell of this system can become manifest as leukemia. For example, a neoplasm of the neutrophilic cells is called a *granulocytic* or *myeloid leukemia*, a neoplasm of lymphocytes is called a *lymphocytic leukemia*; and a neoplasm of erythrocytes is called an *erythroid leukemia*.

Ligation is the formation of a phosphodiester bond to link two adjacent bases separated by a nick in one strand of a double helix of DNA. (The term can also be applied to blunt-end ligation and to joining of RNA.)

Linear energy transfer (LET) is the average amount of energy lost per unit of distance traveled by an ionizing radiation.

Low LET is a radiation characteristic of electrons, X-rays, and gamma rays.

High LET is a radiation characteristic of protons or fast neutrons and alpha particles.

Average LET is specified to even out the effect of a particle that is slowing down near the end of its path and to allow for the fact that secondary particles from photon or neutron beams are not all of the same energy.

Linear hypothesis is the assumption that a dose-effect curve derived from data in the high dose and high dose-rate ranges may be extrapolated through the low dose and low dose-rate range to zero dose, implying that, theoretically, any amount of radiation will cause a dose-proportionate amount of damage.

Linkage describes the tendency of genes to be inherited together as a result of their location on the same chromosome; measured by percent recombination between loci.

Locus is the position of a chromosome at which the gene for a particular trait resides; locus may be occupied by any one of the alleles for the gene.

Malignancy is an essential property of many kinds of cancer cells, which is demonstrated by their ability to proliferate indefinitely, to invade surrounding tissue and to metastasize to other organs.

Marker (DNA) is a fragment of known size used to calibrate an electrophoretic gel.

Marker (genetic) is any allele of interest in an experiment.

Meiosis occurs by two successive divisions (meiosis I and II) that reduce the starting number of $4n$ chromosomes to $1n$ in each of four product cells. Products may mature to germ cells (sperm or eggs).

Metastasis is the spread of cells from a primary tumor to a noncontiguous site, usually via the bloodstream or lymphatics, and the establishment of a secondary growth.

Mitotic delay is delay in passage of a cell through its growth cycle that is induced by radiation.

Mitosis is the division of a eukaryotic somatic cell.

Modified bases are all those except the usual four from which DNA (T, C, A, G) or RNA (U, C, A, G) are synthesized; they result from postsynthetic changes in the nucleic acid.

Mutagens increase the rate of mutation usually by causing changes in DNA.

Mutation is a change in one or more of the DNA bases in a gene. Changes can include insertion of extra bases or deletion of a base(s) or the entire gene. Mutations in coding exons lead to altered protein products; mutations in noncoding regions can lead to altered amounts of protein.

Myeloma is a neoplasm of immunoglobulin-producing plasma cells derived from a B lymphocyte.

Myeloproliferative disorders are diseases involving the abnormal proliferation of various bone marrow cells and are related to the leukemias. They represent a spectrum of bone marrow disorders ranging from non-neoplastic, bone marrow fibrosis and hematopoietic cell dysplasias (which can be fatal) to the leukemias. These disorders are well known after radiation exposure.

NCRP, National Council on Radiation Protection and Measurements.

Necrosis is death of cells which often occurs in solid tumors leading to areas containing degenerating cells.

Neoplasms are, by definition, new growths of cells. They are proliferations of cells, which are no longer under the normal physiological growth control of the body, and that exceed the growth of the normal tissues from which

they are derived. Neoplasms may be benign or malignant (cancerous). In the latter case, they may spread by invasion or

Nondisjunction describes the failure of chromatids (duplicate or duplicated chromosomes) to go to opposite poles of a cell during division (mitosis or meiosis) with the result that one daughter cell has both copies of a chromosome and the other has none.

Northern blot analysis is a technique for determining the presence of specific messenger RNA sequences in cells. Messenger RNA molecules are separated by electrophoresis and then blotted onto a matrix. A radiolabelled probe containing DNA sequences (cDNA), or RNA sequences complementary to the RNA that is to be detected, is applied to the blot and allowed to hybridize. The labeled cDNA is then detected by autoradiography.

Nucleic acid is the general chemical term to describe polymers of deoxyribose- or ribose-nucleic acids which, in turn, consist of repeating units of nucleotides, a base-sugar-phosphate molecule.

Nuclear matrix is a network of fibers surrounding and penetrating the nucleus.

Nude mouse is a mouse which congenitally lacks a thymus and hence mature T-cells. Xenografts of human tumors will often grow in such animals. For unknown reasons these mice are also hairless, hence the term "nude."

Oncogene is a gene whose protein product may be involved in processes leading to the neoplastic transformation of a normal cell.

Operator is the site on DNA at which a repressor protein binds to prevent transcription from initiating at the adjacent promoter.

Operon is a complete unit of bacterial gene expression and regulation, including structural genes, regulator gene(s), and control elements in DNA recognized by regulator gene product(s).

Person-rem is the unit of collective population exposure obtained as the product of the average individual dose in a population times the number of individuals in the population. Thus, the number of person-rem contributed by 1 person exposed to 100 rem is equal to that contributed by 100 people each exposed to 1 rem.

Phage (bacteriophage) is a virus that infects a bacterium.

Phenotype comprises the characteristics of a cell or tissue resulting from the expression of specific genes.

Plasmid is a circular piece of DNA which may reproduce separately from chromosomal DNA within cells, bacteria, or other organisms.

Ploidy is a description of the chromosome content of the cell. Normal mammalian cells contain two copies of each chromosome (except for the sex chromosomes in males) and are diploid. Germ cells contain only one copy and are haploid. Cells in tumors often have missing or additional chromosomes (Aneuploidy), and/or may have one or more chromosome aberrations.

Point mutations are substitutions of single base pairs in a gene.

Probe is a cloned gene or fragment of a cloned gene which can be made radioactive and used to detect homologous DNA (Southern blot or *in situ* hybridization) or RNA (Northern blot).

Progression refers to the tendency of growing neoplasms to undergo sequential phenotypic changes over a period of time accompanied, presumably, by the selection of clones of cells that have a growth advantage. This process frequently leads to the progressive development of increasing degrees of malignancy.

Prokaryote is an organism (bacteria) that lacks a nucleus.

Promoter is (a) a compound that may not itself be carcinogenic, but that stimulates or allows the proliferation of initiated cells; and (b) a DNA sequence where transcription initiates. Promoters, in contrast to enhancers, have direction and are always located near the beginning of the first exon.

Protein kinase is an enzyme that catalyses the phosphorylation of proteins. Phosphorylation and dephosphorylation of proteins appear to be major mechanisms controlling enzyme function. Many oncogenes code for protein kinases.

Proto-oncogene is a gene in a normal cell that may be homologous to a viral transforming gene. Some proto-oncogenes encode proteins that influence the control of normal cellular proliferation and differentiation.

Provirus is the DNA copy of the RNA of a retrovirus which is integrated into the chromosomal DNA of a cell.

Radiosensitivity is the relative susceptibility of cells, tissues, organs, organisms, or any living substance to the injurious action of radiation. Radiosensitivity and its antonym, radioresistance, are generally used in a comparative sense.

Rearrangements are changes in the sequence of genes or of DNA sequences within genes that may lead to alteration in their protein products. Rearrangement of genes is important in such processes as the generation of diversity of antibody molecules.

Receptor is a molecule inside, or on the surface of, cells which recognizes a specific hormone, growth factor, or other biologically active molecule. The receptor may serve as a transducer of signals within the cell.

Recessive allele is obscured in the phenotype of a heterozygote by the dominant allele, often due to inactivity or absence of the product of the recessive allele.

Recessive lethal is an allele that is lethal when the cell is homozygous for it.

Recombinant progeny have a different genotype from that of either parent as the result of the combining of their DNA into a single cell.

Recombination repair is a mode of filling a gap in one strand of duplex DNA by retrieving a homologous single strand from another duplex.

Regulatory gene codes for an RNA or protein product whose function is to control the expression of other genes.

Relative biological effectiveness (RBE) of a given type of ionizing radiation is a factor used to compare the biological effectiveness of absorbed radiation doses (i.e., rads) due to one type of ionizing radiation with that of other types of ionizing radiation.

Relative risk is the ratio of the risk in those exposed to those not exposed (incidence or mortality in exposed population to incidence or mortality in the comparison population).

Rem is a special unit of dose equivalent. The dose equivalent in rem is numerically equal to the absorbed dose in rad multiplied by the quality factor, the distribution factor, and any other necessary modifying factors. The rem represents that quantity of absorbed radiation that is equivalent--in biological damage of a specified sort--to 1 rad of 250 kVp X-rays.

Restriction enzymes are enzymes obtained from bacteria which make cuts at specific sequences of 4-8 base pairs in double-stranded DNA.

Restriction fragment length polymorphism (RFLP) analysis is a method which may be used to identify unique DNA sequences on two homologous chromosomes within a cell.

Retrovirus is a virus in which the genome is RNA instead of DNA.

Reverse transcriptase is an enzyme found mostly in retroviruses which catalyses the production of a complementary DNA strand from an RNA strand.

Reverse transcription is synthesis of DNA on a template of RNA accomplished by a reverse transcriptase enzyme.

S phase is the part of the eukaryotic cell cycle during which synthesis of DNA occurs.

Sarcoma is a malignant neoplasm derived from mesenchymal cells (e.g., connective tissue, vascular tissue, bone, etc.).

Screening is the application of a test that may detect disease in a population of individuals who have no symptoms of the disease.

Segregation is the process by which the chromosomes are separated during meiosis.

Sex chromosomes are the chromosomes that are different in the two sexes; usually labeled X and Y. Females have XX, and males have XY.

Sex linkage is the pattern of inheritance shown by genes carried on a sex chromosome (usually the X).

Sievert (Sv) is the international unit (S.I. unit) of dose equivalent. The dose equivalent in Sv is numerically equal to the absorbed dose in Gy multiplied by the quality factor. 1 Sv = 100 rem.

Silent mutations do not change the product of a gene as, for example those that may occur in introns.

Sister chromatids are the copies of a chromosome produced by its replication.

Somatic cells are all the cells of an organism except those of the germ line.

Southern blotting (named for its developer, Earl Southern) describes the procedure for transferring denatured DNA from an agarose gel to a matrix where it can be hybridized with a complementary nucleic acid.

Stem cell is a cell that has the capacity to repopulate the various functional cells within a given tissue.

Stochastic effects are effects whose probability of occurrence in an exposed population (rather than severity in an affected individual) is a direct function of dose. Hereditary effects and some somatic effects, especially carcinogenesis, are regarded as being stochastic.

Structural gene codes for any RNA or protein product, other than a regulator, and may consist of exons plus introns.

Sublethal damage is damage to a cell which may be caused by radiation or chemicals and which may be repaired in a few hours after the treatment. Frequently, repair of sublethal damage is revealed by giving two treatments separated by a variable time interval or by irradiating at a reduced dose rate.

Synchronized cells are a population of cells in which most of them are at a given stage of the growth cycle at any one time and move through the cell cycle as a cohort.

Target theory (hit theory) is a theory explaining some biological effects of radiation on the basis that ionization, occurring in a discrete volume (the target) within the cell, directly causes a lesion that subsequently results in a physiological response to the damage at that location.

Telomere is the structural end of a chromosome.

Threshold hypothesis is the assumption that no radiation injury occurs below a certain dose level because injury is observed only above that level.

Trans configuration of two sites refers to their presence on two different molecules of DNA (chromosomes).

Transcription is synthesis of RNA on a DNA template. The RNA may be subsequently processed to produce messenger RNA, mRNA. *Reverse transcription* is synthesis of DNA from RNA.

Transduction is excision of viral sequences in cells from chromatin together with cellular sequences and their packaging as mature viral particles.

This process provides a method by which DNA sequences may be transferred from one cell to another with the virus acting as an intermediary.

Transfection is the direct transfer of DNA molecules into a cell. Transfection of specific genes is a powerful tool for determining their function.

Transformation of eukaryotic cells generally refers to their conversion to a state of neoplastic growth in culture.

Transgenic animals are created by introducing new DNA sequences into the germ line as the result of their addition to the egg.

Transition is a mutation in which one pyrimidine is substituted by the other, or in which one purine is substituted by the other.

Translation is the process by which messenger RNA (mRNA) directs the synthesis of protein.

Translocation is the displacement of one part of a chromosome to a different chromosome or to a different part of the same chromosome. An example is the translocation between chromosomes 9 and 22 that leads to the appearance of the Philadelphia chromosome in chronic myelogenous leukemia.

Transversion is a mutation in which a purine is replaced by a pyrimidine or vice versa.

Tumor is a swelling or growth, but the term is often used as a synonym for neoplasm. A tumor may be benign (i.e., the cells do not invade or metastasize) or malignant.

UNSCEAR, United Nations Scientific Committee on the Effects of Atomic Radiation.

Western blot analysis is a procedure analogous to Southern and Northern blot analysis which allows the detection of specific proteins. Proteins are separated by electrophoresis and blotted onto a matrix. They are usually identified by autoradiography following binding of radiolabelled antibodies.

Xenograft is tissue that is transplanted from one species of animal into another. Most commonly, this refers to the transplantation of a human tumor into a nude or immune-deficient mouse.

Zygote is produced by the fusion of two gametes; that is, it is a fertilized egg.

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A study was undertaken by a cytogeneticist, a cell biologist, a health physicist, a mammalian biologist, and a molecular biologist to examine the feasibility of reducing the uncertainties in the estimation of risk due to protracted low doses of ionizing radiation. In addressing the question of feasibility, a review was made of current cellular, molecular, and mammalian radiation data of the way in which altered oncogenic properties could be involved in the loss of growth control that culminates in tumorigenesis; and of the significant progress that had been made in the oncogenic characterizations of several human and animal neoplasms. On the basis of this analysis, the study group concluded that, at the present time, it is feasible to mount a program of radiation research directed at the mechanism(s) of radiation-induced cancer with special reference to risk of neoplasia due to protracted, low doses of sparsely ionizing radiation. To implement a program of research, a review was made by the study group of the methods, techniques, and instruments that would be needed. This review was followed by an initial survey of major laboratories and institutions where scientific personnel and facilities sufficient to participate in a significant part of the program are known to be available. A research agenda of the principal and broad objectives of the program is also discussed.

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