BIOLOGICAL EFFECTIVENESS OF NEUTRONS

Research Needs

February 1994

Submitted to

*The Science Panel of the
Committee on Interagency Radiation
Research and Policy Coordination*

Prepared by

*G.W. Casarett, L.A. Braby, J.J. Broerse,
M.M. Elkind, D.T. Goodhead,
and
N.L. Oleinick*

MASTER

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COMMITTEE ON INTERAGENCY RADIATION RESEARCH
AND POLICY COORDINATION
1019 Nineteenth Street, NW, Suite 700
Washington, D.C. 20036

May 31, 1994

Dr. Alvin L. Young
Chairman, CIRRPC
Office of Agricultural Biotechnology
U.S. Department of Agriculture
14th and Independence Avenue, S.W.
Washington, DC 20250-2200

Dear Dr. Young:

As Chairman of the Science Panel of the Committee on Interagency Radiation Research and Policy Coordination (CIRRPC), I am pleased to provide you with the final report on the *Biological Effectiveness of Neutrons: Research Needs*. This report was written by an independent, multidisciplinary, knowledgeable task group of eminent scientists. It was prepared for the CIRRPC Science Panel in response to a request from the Department of Energy to provide a conceptual plan for a research program that would provide a basis for determining more precisely the biological effectiveness of neutron radiation with emphasis on endpoints relevant to the protection of human health. This request is consistent with the provision in CIRRPC's Charter that "...[the CIRRPC] Science Panel assist the Committee in preparing an appropriate research agenda on selected radiation issues..."

The CIRRPC Science Panel and knowledgeable scientists from both the United States and European radiation research communities reviewed an earlier draft of the report. The task group carefully considered all of the specific comments received and incorporated such comments as were considered appropriate. Overall, the report received high marks for quality. The Science Panel was provided a prepublication copy of the final report to note any changes made as a result of the review.

The Science Panel considers this report an important contribution to science and recommends that it be made available to Federal agencies and other individuals and organizations interested in planning radiation research agendas.

Sincerely,

Randall S. Caswell, Ph.D.
Chairman, CIRRPC Science Panel

Enclosure: as stated

COMMITTEE ON INTERAGENCY RADIATION RESEARCH
AND POLICY COORDINATION
1019 Nineteenth Street, NW, Suite 700
Washington, D.C. 20036

May 27, 1994

Dr. Randall S. Caswell
Chairman, CIRRPC Science Panel
U.S. Department of Commerce
National Institute for Science and Technology
Building 245, Room C-229
Gaithersburg, MD 20899

Dear Dr. Caswell:

The Subpanel on High-LET Radiation is pleased to transmit the final report on the *Biological Effectiveness of Neutrons: Research Needs*, which was prepared by an international task group for the Science Panel of the Committee on Interagency Radiation Research and Policy Coordination. All of the task group members have concurred with forwarding the report to the Science Panel for publication so it can be used by the Federal agencies in planning their agenda in this particular area of radiation research.

It has indeed been my pleasure to work with such a distinguished group of scientists in developing this important contribution to science.

Sincerely,

Bruce W. Wachholz, Ph.D.
CIRRPC Science Subpanel on
High-LET Radiation

Enclosure: as stated

**TASK GROUP ON RESEARCH NEEDS
RELATED TO THE BIOLOGICAL EFFECTIVENESS OF NEUTRONS**

GEORGE W. CASARETT*, Ph.D., *Chairman*, University of Rochester Medical School,
Rochester, New York

LESLIE A. BRABY, Ph.D., Battelle Pacific Northwest Laboratory, Richland, Washington

JOHANNES J. BROERSE, Ph.D., Institute of Applied Radiobiology and Immunology, TNO,
Rijswijk, The Netherlands

MORTIMER M. ELKIND, Ph.D., Department of Radiological Health Sciences,
Colorado State University, Ft. Collins, Colorado

DUDLEY T. GOODHEAD, Ph.D., Medical Research Council, Radiobiology Unit Harwell,
Didcot, United Kingdom

NANCY L. OLEINICK, Ph.D., Department of Radiology, Case Western Reserve University,
Cleveland, Ohio

ORAU Technical Liaison

DIANE S. FLACK, M.S., Technical Advisor

*Deceased

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DEDICATED
TO
DR. GEORGE W. CASARETT

The task group dedicates this report to Dr. George W. Casarett, who served as Chairman of the group and a co-author until his death. This report is the last of over 200 publications and numerous other contributions Dr. Casarett made to the fields of radiation pathology, radiobiology, radiation carcinogenesis, risk analysis, and radiation protection. His expertise in radiobiology, especially in radiation pathology, was recognized by his membership in and appointment to numerous prestigious scientific committees and organizations, including the National Council on Radiation Protection and Measurements, the National Research Council/National Academy of Sciences' Committee on the Biological Effects of Ionizing Radiations, and the International Commission on Radiological Protection. His pursuit of scientific knowledge, dedication to teaching, professional relationships with his colleagues, and devotion to his family serve as examples for his colleagues and friends in both the radiobiology research and radiation protection communities, including those of us who were fortunate enough to have worked with him on the development of this report.

ACKNOWLEDGMENTS

The task group expresses its appreciation to the Committee on Interagency Radiation Research and Policy Coordination for supporting the development of this report on research needs related to the biological effectiveness of neutrons. Comments provided by representatives of the Federal agencies and non-Federal scientists on a draft of the report were invaluable in producing the final report.

The Oak Ridge Associated Universities' Washington Office staff also deserves special acknowledgment for their assistance in accomplishing this task. Particular appreciation is extended to Delores Bigby and Brenda Campbell for preparing the many drafts of this report and to Mary Grace L. Belcher for her assistance in editing the final report.

BIOLOGICAL EFFECTIVENESS OF NEUTRONS

Research Needs

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PREFACE

Readers should note that the major part of the material in this report was submitted prior to distribution of a June 1991 draft report for widespread review by Federal agencies and non-Federal scientists. In February 1992 the task group met to discuss and incorporate the reviewers' comments. Although February 1994 is the publication date of this report, this does not imply that the scientific discussions and data contained within the report are current, as of that date.

Despite the delay in the publication of this scholarly report, the majority of it is still applicable, and the report is considered to be an important contribution to the question of what research is needed to better understand the biological effectiveness of neutron irradiation.

Diane Flack
ORAU/CIRRPC Technical Liaison

SUMMARY

The Committee on Interagency Radiation Research and Policy Coordination (CIRRPC) was established on April 9, 1984 and chartered under the Federal Coordinating Council for Science, Engineering and Technology, Office of Science and Technology Policy, Executive Office of the President. Its overall charge is to coordinate radiation matters among agencies, evaluate radiation research, and provide advice on the formulation of radiation policy. Technical assistance to the Committee is provided by Oak Ridge Associated Universities (ORAU).

This report is in response to the provision in CIRRPC's Charter that "...[the CIRRPC] Science Panel assist the Committee in preparing an appropriate research agenda on selected radiation issues..."

CHARGE AND OBJECTIVES

The charge addressed during the development of this report was to assess research needs related to the biological effectiveness of neutron radiation. More specifically, the goal was to provide a conceptual plan for a research program that would provide a basis for determining more precisely the biological effectiveness of neutron radiation with emphasis on endpoints relevant to the protection of human health.

APPROACH

A group of scientists with expertise in different areas of radiation biology, chemistry and physics were identified by the CIRRPC Science Subpanel on High-LET Radiation (see Appendix A). These scientists, which included members of both the U.S. and European scientific communities, were then requested to evaluate the available scientific information and associated uncertainties regarding the biological effectiveness of neutron radiation and to develop a multidisciplinary research program to narrow the gaps in such knowledge and to reduce the uncertainties that were identified.

TOPICS COVERED IN THE REPORT

This report presents the findings of the experts for seven particular categories of scientific information on neutron biological effectiveness. Chapter II examines the radiobiological mechanisms underlying the assumptions used to estimate human risk from neutrons and other radiations. Chapter III discusses the qualitative and quantitative models used to organize and evaluate experimental observations and to provide extrapolations where direct observations cannot be made. Such models are useful in determining the effectiveness of neutrons relative to other radiations and how this effectiveness varies under different irradiation conditions, for

different species, and for different health effects. Chapter IV discusses the physical principles governing the interaction of radiation with biological systems and the importance of accurate dosimetry in evaluating radiation risk and reducing the uncertainty in the biological data.

The primary energy deposition events from neutron irradiation are translated into biological/cellular damage through chemical and biochemical changes in cellular macromolecules and essential structures. Chapter V deals with the chemical and molecular changes underlying cellular responses and the LET dependence of these changes. Chapter VI, in turn, discusses those cellular and genetic changes which lead to mutation or neoplastic transformation.

Chapters VII and VIII examine deterministic and stochastic effects, respectively, and the data required for the prediction of such effects at different organizational levels (cell, tissue, organ, and animal) and for the extrapolation from experimental results in animals to risks for man. Gaps and uncertainties in this data are examined relative to data required for establishing radiation protection standards for neutrons and procedures for the effective and safe use of neutron and other high-LET radiation therapy.

RESEARCH NEEDS

Research needs for each of the areas discussed above are listed at the end of the appropriate chapter. Chapter IX brings together the essential aspects of research listed in the previous field-specific chapters and provides key comments for the specific research needs listed. The extensiveness of the needed research summarized in Chapter IX reflects the broad scientific aspect of the scope of the charge.

RESEARCH RECOMMENDATIONS AND PRIORITIES

The research plan proposed in Chapter X consists of research recommendations and priorities for four specific research areas: tumorigenesis; mechanisms of neoplastic transformation; mechanisms at low doses; and facilities, resources, and environment. These research priorities are meant to reflect not only the importance of the anticipated research results to the desired improvement in the estimation of human health risks from exposure to neutrons, but also the general aspects of anticipated timeliness of technological progress and research feasibility as the recommended research plan progresses.

Chapter I

INTRODUCTION*

BACKGROUND

The Committee on Interagency Radiation Research and Policy Coordination (CIRRPC) was established on April 9, 1984 and chartered under the Federal Coordinating Council for Science, Engineering and Technology, Office of Science and Technology Policy, Executive Office of the President. Its overall charge is to coordinate radiation matters among agencies, evaluate radiation research, and provide advice on the formulation of radiation policy. This report was prepared in accordance with the provision in CIRRPC's Charter that "...[the CIRRPC] Science Panel assist the Committee in preparing an appropriate research agenda on selected radiation issues..."

CHARGE

The charge addressed during the development of this report was to assess research needs related to the biological effectiveness of neutron radiation. More specifically, the goal was to provide a conceptual plan encompassing a research program that would provide a basis for more precisely determining the biological effectiveness of neutron radiation, with emphasis on endpoints relevant to the protection of human health.

The specific objectives in the charge were:

- (1) to evaluate information and associated uncertainties regarding the biological effectiveness of neutron radiation;
- (2) to formulate a multi-disciplinary research program to narrow the gaps in such knowledge and to reduce the uncertainties which were identified; and
- (3) to develop a plan to implement the program.

The CIRRPC Science Subpanel on High-LET Radiation (Appendix A) tasked a group of experts from both the U.S. and European research communities to address the above objectives. In response to the charge, this report was developed by the experts, with input from numerous other professionals in the international scientific community.

* The major part of the material in this report was prepared prior to June 1991.

RADIOBIOLOGY IN RADIATION PROTECTION

Improvements in systems of radiation protection, and other pragmatic uses of radiobiological information, will depend upon advances in the knowledge of how the biological effectiveness of radiations of different qualities depend on dose and dose rate. Proposed mechanisms must be compatible at least with the observed influence of radiation quality on effect. Furthermore, knowledge concerning the mechanism of the induction of cancer by radiation quite likely will contribute to the broader issue of how cancer is induced by other environmental agents.

Neutron radiobiology is an integral part of radiobiology and, therefore, must be studied in the broader context of the overall field. Among the more important consequences of enlarging our understanding of radiobiology is the likelihood that methods and strategies can be devised for protection against the deleterious effects of ionizing radiation. However, a distinction must be made between basic scientific information and practical formalisms which have been devised for the practice of radiation protection.

The primary purpose of this report is to outline the research needed to understand the biological effectiveness of neutrons, rather than to assess specific problems which may be associated with radiation protection systems in current use. However, if the research goals identified in this report are achieved, information will be forthcoming which will enable improvements to be made in the current practice of radiation protection relative to neutrons.

In the practice of radiation protection, it has been necessary, and/or operationally convenient, to make a number of assumptions about the probable results of ongoing epidemiological studies; the relevance of the data and endpoints derived from biological experimentation; the forms of the dose-effect relationships to be employed; the appropriate methods for data analysis; and the appropriate biophysical models to predict the magnitude of the effects from low, protracted doses. Additional assumptions are included in the models to extrapolate risk across species. These assumptions reflect the inadequate knowledge of the mechanisms involved, including the uncertainties of the relative biological effectiveness and dosimetry issues for various kinds of radiation, the means of assessing the hazards from two or more exposures to radiations of different qualities, and the interactions between radiation and other environmental agents. The need to make such assumptions reflects gaps in our knowledge and, hence, the areas where new research is needed.

RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)

The concept of the *relative biological effectiveness* (RBE) of a given radiation is essential for the evaluation of existing radiobiological data. During much of the history of the study of health physics, RBE had two meanings. This report uses RBE as it has been used in radiobiology to mean the ratio of the doses of two different radiations which produced the same effect. Both radiations had to be specified, as well as the doses, the dose rate, the overall exposure time, the biological system, the endpoints, and the magnitude of the effect.

In addition to the basic meaning of RBE, a second meaning evolved in the practice of radiation protection. This meaning was expected to connote a single specific value for each type of radiation which would reflect the variety of measured values for endpoints relevant to radiation protection. Comments recorded during the 1949 Tripartite Conference (Taylor 1984) indicate that the reference radiation to which radiations of higher biological effectiveness were to be compared was gamma radiation from radium. However, the reference radiation was subsequently changed to orthovoltage x rays (Tripartite Conference 1953). Although it was recognized that the relative effectiveness of gamma rays, compared to orthovoltage x rays, would be less than one, a separate value was not given for gamma rays. In 1950, the recommendations of the International Commission on Radiological Protection (ICRP 1951) included the relative effectiveness values agreed to in the Tripartite Conference of 1949. These early considerations led to the development, and subsequent application, of several concepts and procedures for radiation protection by the National Council on Radiation Protection and Measurements (NCRP 1954) and the ICRP (1955). The meaning of the term "RBE" noted above was subsequently replaced by the quality factor, Q , to make clear that judgement was to be applied, in addition to the experimental data, in determining this value. Thereafter, RBE was to be reserved for the actual radiobiological measurements.

In a 1963 report of the RBE Committee of the ICRP and the ICRU (ICRP/ICRU 1963), the experimentally derived quantity RBE was defined as the *inverse ratio of the absorbed dose of a test radiation to the absorbed dose of a standard, or reference radiation, which produces the same degree of effect* under the same experimental conditions. For purposes of radiation protection, the standard radiations were designated to be x rays, gamma rays, and electrons or positrons of any specific ionization; accordingly, a quality factor of unity was assigned to any radiation having an average LET, in water, of $3.5 \text{ keV}/\mu\text{m}$ or less. Based on this definition, experimentally derived RBE values were associated with particular LET values. The RBE Committee drew attention to evidence that the protraction of doses of a low-LET radiation usually caused a reduction of effect. It was also noted that, because of the differences in the shapes of dose-effect curves, RBE values could increase with decreasing dose to a (presumed) maximum value at minimally low doses, designated RBE_M , if the initial portions of the dose-response curves of both the high- and the low-LET radiations were linear.

According to current conventions (ICRU 1986), the relative biological effectiveness $\text{RBE}(p)$ of radiation B with respect to radiation A (the reference radiation) at a given level of effect probability, p , is defined as:

$$\text{RBE}(p) = \frac{D_A(p)}{D_B(p)}$$

where $D_A(p)$ and $D_B(p)$ are, respectively, the absorbed doses of radiations A and B that cause the effect probability, p . In this context, the term "effect" refers to equal nature, equal severity, and equal frequency. Low-LET radiations, such as orthovoltage x rays and gamma radiation, have been used as the reference radiation to derive neutron RBE values. For the assessment of

the RBE, the time-dependence of the dose administration of radiations A and B is also of predominance. In statements on RBE values for fast neutrons it is relevant to consider the type of low-LET reference radiation because there may be differences in the effectiveness of different low-LET radiations.

The investigation of the parameters affecting the relative biological effectiveness of various radiations under different conditions of exposure can be a fruitful means of pursuing mechanisms of biological effects, as well as degrees of risk. In recent decades, there has been a marked increase in studies of relative biological effectiveness. In some instances, complete dose-response curves have been compared, but, in others, measurements have been limited to doses which yield the same degree of effect or, in some instances, to the effects produced by the same doses.

The earlier values of the RBE for neutrons, which underlie the current value for Q, were based largely on single, moderate-to-high doses of either x or gamma rays, delivered at high dose rates, on the assumption that these low-LET radiations were biologically equivalent. However, with the more widespread use in neutron research of protracted low doses of gamma rays as the reference radiation, higher RBE values have been reported. The latter situation may be due, in part, to a lesser effectiveness of low-dose-rate gamma rays versus low-dose-rate orthovoltage x rays. Additional studies of the comparative biological properties of x and gamma rays are needed to resolve this point.

BIOLOGICAL EFFECTIVENESS OF NEUTRONS

Until a few years ago it was thought that substantial information existed for the spectrum of neutrons released by the atomic bomb detonated over Hiroshima. However, the reassessment of the dosimetry connected with that event suggests that the neutron contribution to the total dose may have been too small to provide data on effects specifically due to neutrons (Kopecky and Preston 1988). The elimination of this source of human data relative to neutron effects has enhanced the importance of data from other sources, such as cellular and animal experimentation.

Neutrons of different qualities have been studied in many experimental systems and animal species for a broad range of biological endpoints. The data have yielded estimates of the RBE of neutrons compared to other ionizing radiations which vary over two orders of magnitude, depending on the energy spectrum of the neutrons used, the doses and dose rates used, the biological system and endpoint studied, and the reference radiation used. Consequently, even for a given radiation and reference radiation, RBE may have many values depending on the particular conditions which may apply. The proceedings of a number of symposia and workshops, as well as numerous review and research articles, have dealt with this subject (e.g., Broerse and Gerber 1982; Sinclair 1983, 1985; U.S. DOE 1985; ICRU 1986; Myers and Johnson 1986; Dennis 1987).

Appendix D in ICRU Report No. 40 (1986) contains a review of the data, some of which are summarized in Table D-4 of that report and reproduced in Table I-1 below. The table lists RBE_M values for various endpoints. Relative to fractionated gamma rays, the data show that

RBE_M values for fission-spectrum neutrons varied from < 10 to > 100, with many values in the range of 15-70.

Table I-1. RBE_M for fission (or optimum energy^a) neutrons versus fractionated gamma rays^b (from ICRU 1986).

Endpoint	Range of RBE _M Values	
Tumor induction	~ 3	- ~ 200
Life shortening	15	- 45
Transformation	35	- 70
Cytogenetic effects	40	- 50
Genetic endpoints in mammalian systems	10	- 45
Other endpoints		
Lens opacification	25	- 200
Micronucleus assay	6	- 60
Testes weight loss	5	- 20

^a "Optimum energy" is the most biologically effective energy.

^b Implied where not actually measured.

A difficulty in deriving reliable and generalizable estimates of RBE is the requirement for appropriate dose-effect curves for the radiations being compared. In the scientific literature on the subject, the influence of radiation quality on a biological effect is usually, although not always, discussed in terms of relative biological effectiveness in one context or another. In some instances, isolated doses were involved for one or both of the radiations being compared, that is, adequate, complete dose dependencies were not determined. Where essentially equal effects happen to occur, RBE values can be estimated for the specified conditions involved. In some instances, where equal effects were not produced in such limited dose-point studies, differences in degrees of effect for equal or comparable doses have been specified. Although the foregoing does not permit the extraction of an RBE, the information may still be of value under certain conditions.

DOSE-RATE DEPENDENCIES

Earlier research on this subject emphasized moderate-to-large doses delivered at relatively high dose rates. Such exposures are relevant to some radiation exposure accidents and to the use of neutrons in radiation therapy for cancer. In recent years, however, there has been increased emphasis on the effects of low doses, and of larger doses at low dose rates, in order

to gain data of greater relevance to the doses and the temporal modes of exposure encountered most frequently in radiation protection.

Among the important unsettled issues in the determination of RBE values for low doses is the dependence of the effectiveness of different radiations on dose size and dose protraction. Dose-rate dependencies are marked in the case of low-LET radiations, but usually less so, when evident at all, in the case of high-LET radiations. Furthermore, the influence of dose rate may depend on the magnitude of the dose, and the direction of the dose-rate dependency may depend upon the quality of the radiation.

The dependencies on dose rate (or dose protraction) and the relative effectiveness of a high-LET radiation, like neutrons, compared to a low-LET reference radiation, are illustrated in Figure I-1. Here, "effect" represents any radiation-induced endpoint, such as lethality, mutation, neoplastic transformation, tumor induction, and so on. At high dose rates, the high-LET curve for small doses usually rises more steeply than the low-LET curve and may, in some instances, drop toward zero effect in a range of doses that is smaller than for a low-LET radiation. It is likely that a maximum in the induction of neoplastically transformed cells *in vitro*, or in tumorigenesis, is reached prior to increased killing of the target cells, which results in a decreased expression of the endpoint (e.g., Han and Elkind 1979; Upton et al. 1970; and Boice et al. 1988, respectively). Reducing the dose rate of a low-LET radiation usually results in a reduced effect; in some instances, the slope of the low-dose-rate curve is less than the initial slope of the high-dose-rate curve (e.g., Han et al. 1980; Thomson et al. 1981; Upton et al. 1970). In contrast, protracting the exposure of a high-LET radiation may result in little or no change in effect (e.g., Hieber et al. 1987; Ullrich 1984; Thomson and Grahn 1988; Terzaghi-Howe 1989; Huiskamp et al. 1989), in an enhanced response (e.g., Hill et al. 1985; Hill et al. 1984; Thomson et al. 1981; Ullrich 1984; Huiskamp et al. 1989), or in a reduced response (e.g., Storer et al. 1979; Fry 1982; Ullrich 1984; Huiskamp et al. 1989). This phenomena is discussed in greater detail in Chapter VI.

In Figure I-1, if the region of small doses is considered with simple interpolation through the region of no data, it is evident that the RBE: will be greater than one when high dose rates are used for both radiations; may increase if the low-LET radiation is delivered in a protracted fashion and the initial slope of the latter curve decreases; and may increase even further if reducing the rate of exposure to the high-LET radiation increases the initial slope of the curve. In each case, if the dose-effect curves have nonzero initial slopes, the RBE will approach a maximum value, RBE_M , as the dose is decreased.

These generalizations have been demonstrated most easily in relatively simple cell systems with which single specific effects can be measured over wide ranges of dose and dose rate, with minimal complication from competing effects. In more complex systems: direct confirmation is more difficult; fewer data are available because of the cost of such research; and competing effects, or complications, may alter the shapes of dose-effect curves, at least for moderate to high doses. For example, for a complex endpoint like cancer induction, dose-effect curves for given types of cancers may be different when only the tissues of tumor origin are irradiated, as opposed to whole body irradiation of the animal.

In many systems, the slopes of the low-dose regions of a dose-effect curve cannot yet be inferred accurately enough simply by extrapolation from data at high doses. Improvements in

the reliability of such extrapolations require an increase in experimental observations and more information on the factors that may influence radiation effectiveness at low doses and low dose rates. Advancement in our knowledge of molecular and cellular mechanisms would also improve our confidence in the accuracy of associated RBE values.

Figure I-1. Schematic dose-response curves for incidence of effect in relation to dose and dose rate of high-LET and low-LET radiation. The box near the origin indicates the region where there are no data.

RESEARCH NEEDS

Thorough experimental investigations of the biological effectiveness of neutrons of various spectra, as well as of monoenergetic neutrons, are needed with particular emphasis on those sources of neutrons which are of importance in radiation protection. As discussed above and in the following chapters, the principal reasons for these needs are that:

- (1) a significant number of workers are currently exposed to neutrons in the course of their occupations, and it is likely that people engaged in space activities will also be exposed to neutrons;

- (2) it is highly unlikely that an adequate base of human epidemiological data will become available on the effects of neutrons or other high-LET radiations; and
- (3) there is a significant likelihood that principles gained from radiobiological research with neutrons will advance our general understanding of the biological effects of high-LET radiations from external sources or internally deposited radionuclides.

The proposed research plan consists of recommendations for additional research and priorities for specific research areas. Chapters II through VIII identify research needs in the areas of physics and dosimetry, modeling, chemical and molecular mechanisms, cellular effects, nonstochastic effects in tissues and organs, and late effects in populations, including carcinogenesis, life span shortening, and hereditary effects. Chapters IX and X summarize the needed research and list prioritized research recommendations, respectively. The priorities reflect not only the importance that the results would have in improving the accuracy of estimates of risk, but also the anticipated length of time for technological advances to make the research feasible. Implementation of the recommendations is left to the appropriate funding agencies.

Chapter II

RADIOBIOLOGICAL PRINCIPLES*

It is usually assumed that the tumorigenic effects of radiation arise as relatively rare stochastic consequences of damage to individual cells. Insult from ionizing radiation is always delivered in the form of discrete tracks of charged particles traversing the cells. Consequently, the initial radiobiological processes may be described in terms of damage to particular target material using general concepts of target theory. In its general form, target theory assumes that the observed all-or-nothing effect is caused by one or more radiation tracks passing through the cell and directly or indirectly causing particular damage to critical components within it. Almost all biophysical models of radiation action incorporate at least some essential concepts of target theory. Model descriptions of the radiobiological mechanisms are usually constructed on selected assumptions and deductions from those assumptions.

The concepts outlined in this chapter are most readily applied to single all-or-nothing radiation-induced changes to individual cells. Tumorigenesis is a more complicated process, involving multiple changes, as well as tissue or host influences, resulting from radiation and other agents or occurring spontaneously. Nevertheless, the general concepts of target theory provide underlying principles commonly used in studying the mechanisms of stochastic effects, including tumorigenesis, and in estimating risks at low doses and low dose rates. In applying these principles, the complex multi-stage nature of tumorigenesis should not be overlooked. Most of what follows in this chapter is directly applicable to single-stage changes, which may well be the rate-limiting steps for radiation tumorigenesis at low doses. In addition, some indications are given of complications that may arise for multi-stage changes, particularly at higher doses.

SINGLE-HIT TARGET THEORY

In the simplest form of target theory, a direct "hit" (i.e., one or more ionizations) of any type in a critical component by a radiation track is assumed to lead, with certainty, to the observable effect in that cell. In this case, the frequency (f) of affected cells in an irradiated population of cells should increase with dose (D) according to the probability of a cell receiving one or more critical hits. Assuming that the hits occur randomly according to a Poisson distribution, then the frequency of cells with one or more hits is:

$$f = 1 - e^{-n} = 1 - e^{-\lambda D} \quad (1)$$

where n is the mean number of critical hits per cell at dose D , and λ is the mean number of critical hits per cell per unit dose.

* The major part of the material in this report was prepared prior to June 1991.

For small n (i.e., low frequency effects and/or low doses) the dose response is approximately linear, with:

$$f \approx \lambda D \quad (2)$$

At higher hit frequencies the dose response takes the form of equation (1), which saturates at high frequency because after the first critical hit in a cell subsequent hits in it cannot lead to additional effects. If the lack of an effect is being measured, such as the frequency of surviving cells (i.e., cells *without* a critical hit), then the dose response is:

$$f' = 1 - f = e^{-\lambda D} \quad (3)$$

This very simple form of target theory may be applicable to the inactivation of many molecules in the dry state and to some viruses, but, in general, it is not appropriate for repair-competent microorganisms and mammalian cells because of their well-established capacity to repair radiation damage.

More refined forms of the single-hit target theory could include the variable probability of effect, depending on the type ("severity") of a hit and on cellular repairability of the damage, and could also include extension of the size of the target for indirect effects. Provided that the tracks act totally independently of one another in regard to each of these processes, the dose response should still conform to equations (1) to (3) (Lea 1955). The numerical value of λ should now be modified to reflect the combined probability, per cell and per unit dose, of all these single-track processes leading to final effective damage. Indirect effects should increase the value of λ , while biochemical repair should reduce it. Therefore, experimental observation of linear or exponential dose responses does not, of itself, indicate that damage cannot be modified or repaired by the cell. Linear or exponential dose responses, as above, are perfectly consistent with any amount of repair, provided that the efficiency of the repair processes acting on the damage from each given track is totally independent of the presence, or absence, of other tracks (i.e., the efficiency of repair is dose independent). The effect of such repair processes is simply to reduce the number of effective hits, which should still follow a Poisson distribution, but with a reduced mean.

MULTI-TRACK EFFECTS

Further extension of the concepts of target theory can consider additional contributions due to multiple tracks, which may increase or decrease the probability with which the damage from single tracks leads to the effect.

The ways in which multiple tracks could *increase* the probability of effect include:

- (1) reduction, by overall burden of damage, in efficiency of cellular repair of individual points of damage (e.g., partial saturation of repair processes, or induction of damage-fixation processes);
- (2) interaction or interference between points of damage to make them less repairable (e.g., formation of exchange aberrations); and
- (3) production of a series of independent changes which together increase the overall probability of final effect (e.g., multiple steps in full neoplastic transformation).

By contrast, *decreases* in probability of effect by multiple tracks could occur by:

- (4) enhancement of cellular repair (e.g., induction of additional repair capabilities); and
- (5) elimination of some of the cells from the population by transferring them to a state in which the effect cannot be expressed (e.g., loss of cell viability).

Other processes, such as multi-track perturbation of the cell cycle, have the potential either to increase or to decrease the probability of effect.

A simple mathematical extension of equations (1) to (3) to include multiple-track effects may be done by means of a general polynomial. Therefore, the previous equations are replaced by:

$$f = 1 - e^{-(a_1 D + a_2 D^2 + \dots)} \quad (4)$$

in general,

$$f \approx a_1 D + a_2 D^2 \dots \quad (5)$$

for low frequency effects, and

$$f' = e^{-(a_1 D + a_2 D^2 \dots)} \quad (6)$$

for lack of an effect (e.g., cell survival).

Attempts to interpret and apply the coefficients $a_1, a_2, \dots$ must usually rely on particular assumptions of radiobiological mechanisms. Many investigations, including experiments, theory and model formulations, are aimed at identifying which assumptions may be most reasonable under given circumstances. Without such radiobiological assumptions, the coefficients provide no more than fitted values which may be valid only in the limited range of the experimental data themselves.

When the equation is to describe a low frequency effect, such as tumorigenesis, arising from a given initial population of cells, it may be convenient to separate out the influence of radiation-induced loss of cell viability by replacing equation (5) with:

$$f \approx (a_1 D + a_2 D^2 + \dots) S(D) \quad (7)$$

where $S(D)$ is the fraction of cells which survive dose D ; this may be described by the form of equation (6).

It is frequently found in practice that only the first two terms of the polynomials in equations (4) to (7) are needed to provide statistically acceptable fits to the experimental data. Most data are fitted, therefore, without including powers of dose greater than D^2 . This simplification may be reasonable for radiobiological mechanisms underlying some of the possible multi-track processes listed above, particularly (1) and (2). However, the assumption of 2-term polynomials is unlikely to be adequate to describe and interpolate over the full dose response if it includes processes (3) and (4).

LOW DOSES AND LOW DOSE RATES

The dose response for an effect arising solely from *single* tracks, acting independently, should conform with equation (1) and should be simply linear with dose (equation [2]) if the frequency of effect is small. It should extend linearly down to zero dose with no threshold because reducing the dose simply proportionately reduces the number of tracks and, therefore, the frequency of effect. The dose response should be independent of dose rate because the time interval between tracks is irrelevant if the tracks are acting totally independently.

Deviations from the form of equation (1) must be due to the effect of *multiple* tracks. These deviations, however, need not be obviously apparent over all portions of the dose response. Hence, apparent linearity over an experimentally accessible portion of the response does not guarantee that only single-track processes are involved or that extrapolation to lower doses is linear. In general, it is expected that multiple-track processes may depend on dose rate as the mean time interval between tracks is varied. Referring to the examples above, simple expectations are that a reduction of dose rate would reduce the effectiveness of radiation acting via processes (1) and (2). Predictions for the other processes are less clear because they are likely to depend on the timings of the particular processes in relation to the intervals between tracks and the overall irradiation time. For most processes, it may be expected that at very low

dose rates multiple-track effects will become negligible because the tracks become effectively independent in time; in this limit, the dose response should conform to equations (1) to (3).

Available experimental and epidemiological data on radiation tumorigenesis can be considered in terms of three regions of the dose response. These are illustrated in Figures II-1 and II-2 by schematic dose-response curves, consistent with the form of equation (7), for the frequency of an effect, such as a type of tumor, induced by neutrons or gamma rays. Figure II-1 shows the response plotted against absorbed dose on linear scales. Figure II-2 shows the identical curves again plotted (upper abscissa) against absorbed dose, but on logarithmic scales to magnify the lower dose region, and also plotted (lower abscissa) against the mean numbers of tracks per cell nucleus (assuming spherical nuclei of $8\ \mu\text{m}$ diameter for these illustrations).

Figure II-1. Schematic dose-response curves for induction of stochastic biological effects by high-LET (e.g., fission neutron) and low-LET (e.g., gamma ray) radiations. The effect could be, for example, tumors, mutations, or chromosome aberrations. The maximum frequencies achievable need not be equal for the different radiations. Dose regions I, II, and III are explained in the text and in Figure II-2. (Modified from Goodhead 1988.)

Figure II-2. The same schematic dose-response curves as in Figure II-1, but replotted on logarithmic axes and showing also the mean numbers of tracks per cell nucleus (evaluated for $8 \mu\text{m}$ spherical nuclei using microdosimetric data [ICRU 1983; Goodhead 1984]). The region of "definite" 1-track action has been chosen to correspond to ~ 0.2 tracks per nucleus because under this condition $< 10\%$ of the traversed nuclei will have received more than one traversal (assuming Poisson statistics [ICRU 1983; Goodhead 1984, 1987]). (From Goodhead 1988.)

In Figures II-1 and II-2 above, the dose scale is divided into three approximate regions, as follows.

Low-Dose Region (I)

In the true "low-dose" region (I), there are so few radiation tracks that a single cell (or nucleus) is very unlikely to be traversed by more than one track. In this region of "definite" single-track action, the dose response for single-cell effects is almost bound to be linear and independent of dose rate. This is because varying the dose proportionately varies the number of cells singly traversed, and varying the dose rate varies only the time between these independent events. These simple expectations would be violated only if the rare multiple traversals very greatly enhanced the probability of effect, such as may be the case if radiation

tumorigenesis *requires* two radiation-induced steps well separated in time. There are virtually no epidemiological or experimental data (particularly in animal or mammalian cell systems) in this region. Therefore, there is almost no direct information about how a cell or a tissue may respond to the damage from a single radiation track.

This is the region of main practical concern in radiation protection. For example, a worker who over a year receives a uniform whole-body gamma-ray irradiation equivalent to an annual exposure limit of 50 mSv (ICRP 1977a), corresponding to 50 mGy ($Q=1$), will have received over the full year an average of about 50 electron tracks through each cell nucleus in his body. Multi-track processes should then be relevant only if they operate over long periods of time, comparable at least to the times between tracks. If, instead, the sample is uniformly irradiated with pure 1 MeV neutrons (and ignoring attenuation and energy degradation in the body) then the 50 mSv limit corresponds to 5 mGy of neutrons ($Q=10$) and an average of about 0.05 tracks (mostly high-LET recoil protons) through each cell nucleus during the year. These tracks must clearly act independently unless multi-track processes persist over very long periods of time, extending to many years. The independence of tracks is even clearer within the more recent ICRP recommendations of reduced occupational limits to an average of 20 mSv per year and Q values of up to 20 for neutrons (ICRP 1991).

In practice, neutron irradiations in the body are always composed of a spectrum of energies and are accompanied by a contribution from gamma rays which usually strongly dominate, through low-LET electrons, the total number of tracks. As can be seen from Figure II-2, if 1% of the absorbed dose is due to gamma rays, this will contribute approximately the same number of tracks per cell nucleus (by electrons) as is contributed by the 99% absorbed dose from neutrons (by protons). Also, the exposure is seldom uniform in the body or in time, and cell nuclei have a variety of sizes and shapes. Nevertheless, the dose and dose-rate region of main practical relevance in radiation protection (0-50 mSv per year) is characterized by small average numbers of tracks per cell with long intervals of time between them. Therefore, the effects are likely to be dominated by individual tracks acting alone.

Intermediate-Dose Region (II)

In the "intermediate-dose" region (II), it is commonly assumed that tracks act independently if a linear term (a_1) is obtainable by curve-fitting to equations such as (4) to (7). However, most of the epidemiological and experimental data used for dose-response curve fitting are usually towards the higher-dose end of this dose region, and individual cells may, in fact, have been traversed by considerable numbers of tracks. The assumption of one-track action for this region considers that the relevant metabolic processes of the cell are not influenced by the additional tracks in any way which could alter the efficiency of these processes and, therefore, the expression of the ultimate biological damage of each individual track. This region of the dose response, then, should be independent of dose rate. On these assumptions, it is conventional to interpolate linearly from this region to zero dose in order to deduce the effectiveness of low dose rates and low doses of radiation, that is, region I. Such interpolation is based on the coefficient a_1 in equations (4) to (7) and the assumption that a_1 remains

unchanged even to very low doses and very low dose rates. There are numerous radiobiological studies, mostly with cells *in vitro*, which suggest that this common assumption may not be universally valid.

High-Dose Region (III)

In the "high-dose" region (III), there are often clearly observable multiple-track processes causing the upward or downward curvature of the dose response, including cooperative effects and competing processes like cell killing. Dependence on dose rate is to be expected. This region is often the most experimentally accessible, particularly for studies of molecular damage. Mechanisms in this region need to be adequately understood and described if such data are to be used for curve fitting and extrapolation, together with data from region II, to the low doses and low dose rates of prime relevance.

RADIATION QUALITY AND RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)

A very wide variety of radiobiological data have shown that high-LET radiations, including neutrons, are more effective than low-LET radiations when a comparison is made of the doses required to produce a given level of effect. This greater effectiveness of neutrons is usually particularly marked in the regions of the lowest doses where data are available, which implies that the individual tracks from neutrons have a higher probability of effect than do a very much larger number of tracks from low-LET radiation. Thus, the concentration of energy deposition within neutron tracks more than compensates for the reduced number of tracks per unit dose.

The RBEs of particular relevance in radiation protection are those which apply in the true low-dose region (I), in which tracks are most likely to act individually. At these minimally low doses the RBEs of given radiations should be constant, independent of both dose and dose rate, because varying the dose varies only the number of cells which are traversed by single tracks. This RBE, at minimal doses, could in principle be calculated by direct comparison of measured effectiveness per unit dose of neutrons and low-LET radiations in the low dose region or from experimental measurements of the effectiveness of single tracks of the radiations. Current experimental methods have not been able to achieve this.

Instead, it is conventional to estimate the RBE at minimal doses as the ratio of the a_1 values of the two radiations, determined by fitting equations such as (4) to (7) to the available data at intermediate and high doses. This method assumes that the multiple tracks in the intermediate dose region do not influence the effectiveness of each other at all and, consequently, that the a_1 values are unchanged right down to zero dose and that they are independent of dose rate. This assumption is best supported for high-LET radiations for which radiobiological data usually show strongly linear dose responses, which vary little with dose rate, including some data which approach the true single-track region. Notable exceptions have been reported, however, in cellular, animal, and human systems (e.g., Hill et al. 1984a; Miller et al.

1990; Storer et al. 1979; Brewen et al. 1979; Thomson et al. 1981; Fry 1982, 1990; Ullrich 1984; Huiskamp et al. 1989; Darby 1990). The assumption of constant a_1 , independent of dose rate, for low-LET reference radiations is also questioned by data from numerous studies on cellular systems (e.g., Brewen et al. 1979; Fertil et al. 1980; Han et al. 1980; Cox 1982; Thacker and Stretch 1983; Strniste and Chen 1985; Sanderson and Morley 1986; Ikushima 1987; Morgan et al. 1987; Shadley and Wolff 1987; Wolff et al. 1988; Marples and Joiner 1989; Kronenberg and Little 1989; Jones et al. 1989; Redpath et al. 1990; Cai and Liu 1990).

DEVIATIONS FROM CONVENTIONAL EXPECTATIONS

The conventional approach to estimating both absolute biological effectiveness and RBE, at minimal doses, is based on the assumption of constant a_1 values from the intermediate dose region down to zero dose and is independent of dose rate. There are, in principle, many ways in which this may be violated.

For single-cell effects it may be violated if there are significant multiple-track processes in the intermediate dose region. Such processes could include, for example, induction of damage-fixation processes or of enhanced repair by small numbers of tracks. There is strong evidence of induction or amplification in nonmammalian systems and some such indications in mammalian cells (Pohl-Rüling et al. 1983; Lücke-Huhle et al. 1986; Sanderson and Morley 1986; Ikushima 1987; Wolff et al. 1988; Cai and Liu 1990). Possibilities which have been suggested to explain observed dose-rate dependence of neutron-induced cell transformation include promotion by multiple tracks or enhancement of misrepair (Hill et al. 1984a), variations of cell sensitivity to transformation and killing with time through the cell cycle (Rossi and Kellerer 1986; Brenner and Hall 1990; Elkind 1991a,b), and induction or enhancement of repair (Goodhead 1988).

Another general way in which the simple conventional expectations would be violated is if the probability of tumorigenesis after an initial radiation-induced change could be significantly modified by additional radiation-induced change(s) at later time(s), such as in multi-step models of tumorigenesis (Moolgavkar 1983). In such a case, even a linear dose response to acute doses could be enhanced (e.g., by clonal expansion due to selective advantage) or depressed (e.g., by selective disadvantage) by reducing the dose rate. A critical question here deals with determining on which step(s) radiation can act in multi-step mechanisms of tumorigenesis and how well it competes with the other causes of these steps.

The general approach described in this chapter would also need appreciable modification if the biological effect of interest required damage to more than one cell or is influenced by damage to additional cells. For example, van Bekkum and Bentvelzen (1982) and Mole (1984) have hypothesized that radiation tumorigenesis involves DNA transfer from one radiation-inactivated cell to an adjacent radiation-damaged cell. In this case, the true low-dose region (I) of action by individual tracks alone would be shifted to even lower doses than in Figures II-1 and II-2 because the volume containing the target must be enlarged to include adjacent cells. This two-cell hypothesis should be experimentally testable with epithermal neutrons, whose individual tracks are too short to hit the nuclei of two adjacent cells (Goodhead 1988).

Some of the above processes allow, in principle, for the possibility of a true threshold in the dose-effect curve, especially for low-LET radiations. The most basic requirement for a true dose threshold is that any single track of the radiation be totally unable to produce the effect. Thus, no biological effect would be observed in the true low dose region where cells are hit only by single tracks. There is little experimental evidence to demonstrate such a situation. Considerable microdosimetric, biochemical, and cellular data lead to the expectation that a single track, from even low-LET radiation, has a finite probability of producing a single-step permanent change (such as a mutation) in a cell. For high-LET radiations, this expectation is particularly strong because of the very substantial initial damage which can be caused by a single track (Goodhead 1992). In this case, a true dose threshold may exist only for an effect which requires more than one such single-step change within the same cell and separated in time, and requires that at least two of these steps must be induced by radiation alone, rather than in competition with spontaneous changes or other agents.

RESEARCH NEEDS

Most of the evidence now used to support the basic assumptions that we make about the effects of ionizing radiation comes from the consistency between predictions based on these assumptions and experimental observations. However, alternative assumptions that would lead to the same predictions have been discussed. Direct tests of the effectiveness of single tracks and of the mechanisms that might lead to multiple-track effects would help eliminate much of the uncertainty underlying development of specific theories of the biological effectiveness of neutrons. Such tests depend in part on the biological systems and endpoints which will be discussed in subsequent chapters, but some basic requirements for such experiments can be enumerated here.

(1) The biological effect should be correlated with the actual number of charged particle tracks initiating it at the cellular or subcellular level. Investigation of the effects of single tracks can be approached by dose protraction of fairly small doses of neutrons to reduce the proportion of multi-track occurrences and to separate them in time. Neutrons are a necessary tool when tissue systems are to be irradiated with uniform doses, but they have a number of deficiencies for basic studies because they always produce a spectrum of secondary particles and they are always accompanied by gamma rays. For basic studies of single-cell effects, directly ionizing particles, representative of components of the spectrum produced by neutrons, are preferred, usually as well-defined monoenergetic track-segment irradiations with protons and alpha particles (e.g., Barendsen et al. 1966; Cox et al. 1977; Bird et al. 1980; Geard et al. 1980; Belli et al. 1991, 1992). Current developments of microbeam facilities are making it possible to apply exact numbers of charged particles to specific locations in individual cells (Braby 1992).

(2) Effects should be assessed preferably for endpoints that correspond to a specific biological change, for example, a particular type of mutation or tumor, rather than

endpoints, such as lethality or life shortening alone, that may be caused by a variety of types of change; each type may have a different dependence on the number of tracks, dose rate, and radiation quality.

(3) Modifying factors, such as induced repair enzymes, nuclear structure, and chemical environment of the DNA within the nucleus, should be investigated independently using mutant cell lines, drugs which modify metabolic processes, and changes in the external environment.

(4) At all levels of study, attempts should be made to establish whether there are *qualitative*, as well as quantitative, differences between effects produced by neutrons as compared to low-LET radiations.

Chapter III

ORGANIZING DATA FOR PREDICTION OF RISKS*

INTRODUCTION

Over the course of years, considerable data have been collected on the biological effects of neutrons and low-LET radiations. These data have involved a wide variety of different endpoints, biological organisms and cells, dose levels, and dose rates. An unequivocal trend toward a single relationship between RBE and any physical property of the radiation has not been found. RBE values for specific pairs of radiations and fixed dose rates, but for different biological systems, cover a wide range. The response of a single biological system exposed to different dose rates and radiation qualities can generally be predicted qualitatively, but accurate quantitative predictions are not possible.

The limitations of our predictive capabilities appear to be related to the diverse nature of the health effects endpoints for which predictions are sought and to the complex nature of the many interacting physical, chemical, and biological processes that lead to those effects. Furthermore, it is not possible to obtain statistically significant epidemiological data for each of the specific types of radiation and dose rates that are of major concern in radiation protection. Thus, it is necessary to use the available human data, in combination with animal and cell data, to elucidate trends and extrapolate to the relevant conditions.

A model of the intervening process is an essential tool in making the connection between observation and prediction. Such models range from simple verbal descriptions of the sequences of events and purely phenomenological equations designed to fit the observed dose-effect relationships, to complex descriptions of physical and biochemical mechanisms and mathematical expressions that are designed to represent the characteristics of specific mechanisms. Figure I-1 and the accompanying discussion amount to a group of phenomenological models for the response of "typical" systems.

In general, phenomenological models can be developed more easily than mechanistic models. Phenomenological models can be used to guide the interpolation of expected effects within the range where observations have confirmed the model and are sometimes used for limited extrapolation. However, predicting effects outside the range where measurement is practical requires an understanding of the underlying mechanisms. An understanding of mechanisms also allows evaluation of the effects of combinations of damage produced by independent agents, minimizing the experimental measurements needed to determine the risks of combined exposures to radiation and other environmental hazards.

An important characteristic of useful models is that they are testable and potentially falsifiable. It is seldom, if ever, possible to verify a model; there are usually alternative models

* The major part of the material in this report was prepared prior to June 1991.

which would predict exactly the same results for any given combination of experimental conditions. Agreement between the predictions of a model and experimental observations only shows that the model is consistent with the actual process. However, if an experimental observation is inconsistent with the predictions of the model, then that model is clearly not a valid representation of the actual process involved. Thus, while it is not generally possible to prove that any one model is correct, it is possible to show that many models are incorrect, and, eventually, to show that the actual mechanisms must fall within a relatively narrow range of options.

It is not essential that a model be quantitative or reduced to a mathematical expression for it to be falsifiable. In many cases different assumptions about mechanisms result in predictions of qualitatively different results that can be tested in relatively simple experiments.

Models are useful at all levels in the biological system and at all stages of the development of the biological consequences. One important function of models is to help clarify the sequence of events that leads from physical damage through cellular effects to tissue and organ effects and, eventually, to provide testable estimates of risk, for example, the risk of oncogenesis. Some of the factors that are known or suspected to influence radiation oncogenesis are shown in Figure III-1. The organization of this figure reflects many assumptions about the sequence of events and represents only one possible approach to modeling the entire health effects process. Most of the emphasis to date has been on the response of individual cells.

Mechanistic models of radiation effects generally begin with specific patterns of energy deposition in subcellular structures, not the description of the incident radiation. Thus, it is unlikely that a specific "neutron effect" model will evolve unless experimental evidence is found that shows unexpected biological consequences of nuclear displacements produced by neutron scattering. More likely, the effects of neutrons will be derived from a set of models that will also apply to other high- and low-LET radiations. Similar energy deposition events in macromolecules can be produced by almost any incident radiation; it is the frequency and spatial distribution of those events per unit dose that varies from one radiation type to another. However, the spatial distributions produced by neutrons may include combinations that are not produced by low-LET radiations. The models discussed here are not specific to neutrons or to any other radiation, but apply to all radiations.

BACKGROUND AND CURRENT STATUS

Effects in Single Cells

Diverse models, utilizing varying degrees of mathematical formalism, have been used to describe the response of cells to ionizing radiation. Most of these models have dealt with mechanisms in that they assume that the response follows a specific form of chemical kinetics, and, as a result, each is limited to mechanisms that proceed with those kinetics. However, the models are usually phenomenological in the sense that they do not attempt to cover the

MODIFYING FACTORS

* May be repair deficient or have the equivalent of preexisting damage.

Figure III-1. An indication of some of the factors that might be included in a comprehensive model of radiation response. The ovals denote stages in the progression to malignancy; the rectangles denote processes leading to the next stage; and the list at the right gives some of the factors that can modify these processes.

mechanisms of all of the processes leading from energy deposition to effect. Instead, they generally assume that a single step in that complex process is rate limiting, and they deal only with the kinetics of that limiting process. All other processes are dealt with as constants.

Mathematical models of cell response, and the understanding of the mechanisms that they convey, have evolved rapidly. Early models attempted to account for the shape of the typical dose-response curve in terms of the number of hits required to inactivate a target or the number of targets that must be inactivated to kill the cell. It is now recognized that models that assume that the response of the cell depends solely on the physical and chemical properties of the initial interaction are inadequate to describe the response of most systems. For example, it is now recognized that the biochemistry of the cell plays a major role in determining the cell response, and models addressing the interaction between the initial damage and the consequent biochemical changes are evolving.

A fundamental assumption of radiation biology is that radiation must interact with a cell in order to affect it. This interaction usually takes the form of an ionizing particle passing through the cell and depositing energy, in the form of ionizations and excitations, within it. The consequences of this assumption were clearly stated by Lea (1955) and have served as the basis of most other models of radiation effect. Simple "hit" theory, based on the concept that a single interaction is sufficient to cause inactivation, results in an exponential dose-response relationship and is very successful in describing the response of passive systems such as dry molecules or viruses. Lea also pointed out that for these simple systems an increase in the LET of the charged particles will result in a decrease in effect per unit dose since the energy will be concentrated in a smaller number of targets.

Shouldered survival curves and increasing effectiveness with increasing LET can be introduced by assuming that a single low-LET track does not deposit enough energy to cause inactivation (multihit model) or that there are two or more entities within a cell that must be inactivated to inactivate the cell (multitarget models). These models, which have been thoroughly described many times (e.g., Elkind and Whitmore 1967), produce curves that can be made to fit acute exposure survival data for typical mammalian cells, but do not provide an understanding of dose rate, fractionation, and cell cycle effects.

In order to address time-dependent aspects of cell survival, models also must include the effects of chemical and biochemical reactions that modify the products of irradiation. Models commonly in use fall into two distinct classes, based on the assumed effect of unrepaired damage remaining in the cell. One group of models, characterized by the dual radiation action model (Kellerer and Rossi 1972, 1978) and the molecular model (Chadwick and Leenhouts 1981), assumes that the initial lesions produced by the interaction of radiation with critical components of the cell (either directly or indirectly through free radicals) are sublethal in nature. That is, they are not lethal unless they combine in some way with additional damage to produce a lethal lesion. The alternative models, typified by the cybernetic model (Kappos and Pohlit 1972) and the saturation models (Goodhead 1985a; Calkins 1971; Sontag 1987), assume that damage produced by radiation events is lethal unless it is repaired.

Under most conditions, both types of models can be used to fit experimental measurements for effects such as mutation and transformation, as well as for the reproductive survival of cells. Furthermore, they can reproduce the effects of dose rate and dose

fractionation (Curtis 1986). However, it appears that there must be a component involving the repair of potentially lethal damage to account for the increases in survival observed when cells are held without division for some time following irradiation. There is also evidence that other types of mechanisms, such as induction of increased repair capacity or effects of radiation damage on the chromatin structure (Wheeler and Wierowski 1983), may contribute to the overall response of cells.

Although it appears that none of the models now available provides a complete description of the response of any cell type to all radiations and irradiation procedures, the testing and comparison of these models help to guide experimental work on the mechanisms and consequences of radiation effects. These models also provide a guide for extrapolation to the effects of doses and dose rates relevant to radiation protection problems. For example, at low doses the simple sublethal damage models result in a quadratic or linear-quadratic dose dependence, while the simple potentially lethal damage models result in a purely linear dependence.

A number of models of a more strictly phenomenological nature have also proven to be useful. For example, Katz (Katz and Sharma 1974) developed a mathematical model of the response of cells to charged particles of different charges and velocities. This model uses a nonstochastic representation of the charged particle track, which has been successfully used in studies of detectors such as photographic emulsions, together with experimental results for the response of cells to x or gamma radiation, to determine the effect of the delta rays produced by high-energy radiations. Models of this type are useful in predicting the effects of accelerator beams and cosmic rays, but they do not provide a means of investigating the effects of dose rate on the response of cells exposed to neutrons and gamma rays.

Another purely phenomenological approach is characterized by the hit size effectiveness model (Bond and Varma 1983). In this model, experimental data on the probability of response of cells exposed to a variety of different types of radiation and experimental data on the distribution of energy depositions produced by each type of radiation in a site of a selected size are used to unfold a response function of this type of cell. Assuming that a uniform sphere, a few micrometers in diameter, is a satisfactory phantom for a cell nucleus, this approach provides a means of estimating effects associated with a wide range of different radiations. It also provides interesting information on the energy required to cause an effect and, therefore, may help in formulating ideas on mechanisms. However, this approach does not directly involve the biochemical mechanisms of the cell and cannot be used to develop tests of theories about those mechanisms.

Response in Tissues and Animals

Less progress has been made in modeling the effects of radiation on intact animals and in relating the effects in cells to consequences at the tissue, organ, and animal levels. It is generally assumed that cancer induction and other detrimental effects originate with damage to individual cells, but it is also evident that a number of other factors enter into the expression of that damage.

Some of the most obvious of these factors are the response of the immune system to altered cells, the regulatory effect of hormones and related systems, the growth rate of the cells of a given tissue, and the state of differentiation of the cells at the time of irradiation. All of these factors are subject to alteration due to irradiation of the animal. For example, irradiation of the whole animal may damage a small number of cells in all tissues. Damage in one of these tissues may stimulate the production of a growth factor which, in turn, alters the response of cells in another organ. Similarly, damage to cells during the early part of a protracted irradiation may stimulate neighboring cells to divide and thus alter their radiation sensitivity. The study of responses at one level of biological organization, with the intention of using the result to predict effects at another level of organization, has been referred to as multi-level research. Research in this area is at an early stage, and predictive models are relatively rare, but various starting points have been suggested.

Although animal studies with sufficient detail and precision to reveal trends in the induction of cancer have been limited because they are time-consuming and expensive, trends are becoming evident in the results of studies that have been done. For example, work with mice exposed to gamma rays at high dose rate (Storer et al. 1988) suggests that for most types of cancer the susceptibility for radiation induction is proportional to the natural incidence of that type of cancer in a given strain and gender. Furthermore, the relative risk coefficients for mice and humans were quite similar for several cancer types. Thus, the essentially phenomenological relative risk model is gaining credibility not only for predicting the risk as a function of age for single species, but also as a way of extrapolating between species. However, the same studies show that other tumor types cannot be treated by this model. A more mechanistic understanding will evidently be required to determine when the relative risk model is appropriate.

Multi-step models of carcinogenesis, originally introduced to explain the observation that the incidence rate of many carcinomas increases with the logarithm of age (Armitage and Doll 1954), have evolved over many years. It is now common to associate the steps with mechanistic concepts such as initiation, promotion, and immortalization. Although most of the work on multi-step models deals with chemical carcinogens, some theories on how radiation might induce the apparent changes are now being considered. Van Bekkum (1975) and Mole (1984) have proposed that initiation is the result of a spontaneous transfer of genetic material between neighboring damaged cells. The activation of specific oncogenes is being considered as a potential mechanism for promotion and immortalization, as well as initiation, but these theories are still in the early stages of development. Similarly, it may be possible for conditions at the outer surface of a cell to affect regulatory systems and bring about the expression of specific genes.

An alternative to multi-stage models, a two-stage model based on antioncogene inactivation, has been developed more recently (Moolgavkar 1986). This model assumes that mutations must occur in homologous antioncogenes. As a result, the shape of the incidence curve is determined by the growth curve for normal cells in the target tissue and the cellular kinetics of cells with one inactivated antioncogene (intermediate cells). The differences in the growth of different tissues then account for the differences in incidence rates for different cancers. Similarly, environmental factors and chemicals may enhance tumor production through their effect on the growth of intermediate cells. This model also accounts well for some forms

of heritable susceptibility. If all of an individual's cells contain an inactivated antioncogene, the probability of both homologous genes being inactivated in some cell is relatively high. Other factors, such as deficiencies in the capacity of cells to repair certain types of damage or differences in the individual's metabolism of some classes of chemical compounds, may alter the probability of gene inactivation. Although this model accounts for many features of carcinogenesis and provides good quantitative predictions in some cases, it is not complete in itself since it does not include factors such as immune response. Also, other mechanisms, such as the activation of oncogenes and altered growth factor production and binding, seem to be important in some tumor systems.

RESEARCH NEEDS

Since models, particularly quantitative ones, are so useful in organizing experimental observations and guiding extrapolations to effects of low doses and different radiation qualities, it is important that the development of new and refined models maintain pace with progress in experimental observations. Models are not normally developed independently of experimental observations. More typically, new observations stimulate new ideas about mechanisms that are then incorporated into the models. Alternative models generally reveal differences that can be tested through specific types of experiments. These experiments often provide additional data that stimulate further development of the models. Thus, there is a continuous feedback process with new theories or mathematical approaches stimulating experiments, and new experimental observations stimulating additional progress in modeling. Furthermore, modeling of radiation effects draws on new concepts and techniques developed in other areas of biology and modeling, just as experimental radiobiology does. Thus, the future directions and research needs in modeling the effects of neutrons depend, in part, on the direction of experimental results. However, there is also a clear goal for modeling studies; a testable model that can be used to evaluate the risks of specific radiation exposures. This well-defined goal provides some criteria for assigning priorities to specific types of modeling.

Phenomenological Models

Phenomenological models have traditionally provided the first step toward a full understanding of many processes in physics and chemistry by organizing experimental observations in ways that allow the recognition of trends and significant variables. Dose-effect relationships for neutrons and other radiations have been modeled in this way, and this has led to a start on mechanistic modeling of cell effects. However, relatively little has been done in the modeling of the differences between established cell lines and normal cells, the susceptibility to tumorigenesis in different tissues, or the differences between cells derived from different species. Similarly, little has been done to categorize end effects, such as specific tumor types, in order to determine if they can be grouped according to characteristics such as the shape of the dose-effect relationship, latent period, dependence on dose rate or specific promoter

functions, or the molecular genetics of the individual. These areas should receive increased attention so that new findings relevant to mechanisms can be formed into a coherent picture.

A great deal of data on a wide range of biological systems, endpoints, and radiation types exist. It is essential, for both economic reasons and to avoid additional delay, that the fullest possible use be made of these existing data. In order to accomplish this, additional effort must be put into making the existing data available in an easily accessed format. Attention should be given to "negative" as well as "positive" results since results which were considered negative with respect to testing one hypothesis may be the evidence needed to establish the suitability of some previously unrecognized model. Furthermore, experiments are sometimes repeated simply because the "negative" result was not reported in a way that allowed future workers in related fields to find it. It may be necessary to establish a national data base on the effects of neutron and related irradiations, similar in operation to the data base projects which gather and organize atomic and nuclear cross section data, and make it available to the research community. The National Radiobiology Archives includes a data base on mammalian animal studies, but it does not include human, cellular and molecular studies (Watson 1993).

One of the critical areas in the application of experimental data to the evaluation of risk estimates for neutron and other radiation exposures is the question of extrapolation from one species to another and from one health effect to another. Although some work has been done on the application of relative risk models to relate effects in different species, a great deal more work in this area is needed. Valuable information on the role of specific physiological processes in altering the expression of specific types of radiation damage is probably hidden in the differences in the response of different strains of mice to specific irradiations. Correlations between the responses of these strains to other environmental challenges (chemical mutagens, pathogens, stress, etc.) and their response to irradiation may provide a starting point for further modeling.

Mechanistic Modeling

For many aspects of the response of biological systems to ionizing radiation, enough is already known to make it feasible to attempt to model alternative mechanisms which may control these responses. Many of these data come from areas outside the study of radiation effects, such as chemical mutagenesis data and molecular genetics. Other areas, particularly in some aspects of effects in animals, may require much more progress in phenomenology before mechanistic studies can be formulated. However, the conclusions of models and risk estimates will always involve some element of uncertainty until all of the mechanisms involved can be adequately described, so these difficult problems will eventually be addressed. One perennial challenge to modelers is to choose problems which are simple enough to be tractable, but are also complex enough to be relevant to the underlying goal of the research.

In recent years significant progress has been made in the understanding of the initial products of the interaction of radiation with biological matter. However, the study of the interaction of these initial products with the complex structures of critical macromolecules has just begun. Since the modeling of the structure and function of macromolecules is advancing

rapidly, it is important to take this opportunity to model the effects of radiation products on the integrity of macromolecules. One of the consequences of radiation damage in chromatin is the production of breaks and deletions in chromosomes. Recent advances in experimental techniques for characterizing the ends of these deletions and the effects of biochemical repair processes provide an opportunity to model these processes as well.

Models of the dose-response relationship in cells, and of the effects of radiation quality and dose protraction on this response, are gradually being enhanced by the inclusion of kinetic features which are believed to be characteristic of biochemical mechanisms (Ostashevsky 1989). Additional work is needed in this area, both to extend the range of biochemical processes which can be described and to compare the predictions of models incorporating different kinetic behaviors with experimental data.

Recent advances in the study of oncogenes, chemical carcinogens, and genetic susceptibility to malignancy provide opportunities to devise and test new models of oncogenesis. A full mechanistic description of oncogenesis is undoubtedly a long term goal, but individual components of this multistep process may be addressed within an overall framework which is largely phenomenological. Emphasis should be placed on those aspects of the overall model which will help in the utilization of cell effects data to estimate risks to humans exposed at low doses and low dose rates to both high- and low-LET radiations. These include the major mechanisms by which damage to cells is expressed as malignant transformation, the nature of promotion and other steps in the process of oncogenesis, and the factors which modify the expression of damage in different tissues and species (Elkind et al. 1990).

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Chapter IV

PHYSICS AND DOSIMETRY*

INTRODUCTION

In any effort to investigate the risks resulting from exposure to various types of radiation, accurate measurement of the radiation is necessary. However, the physics of the energy deposition process may make the measurements difficult or impossible in some situations. Therefore, it is important both to know the level of accuracy needed to make a particular biological experiment meaningful and to understand the underlying physical processes and how they may limit the measurement.

The physical processes of energy deposition by ionizing radiation involve discrete interactions and result in a nonuniform distribution of reaction products through the irradiated material. At "low" doses this discrete energy transfer results in significant random variations in the energy deposited in objects the size of biological cells and organelles.

This chapter will review the physical interactions relevant to the passage of ionizing radiation through biological materials. The consequences of these interactions, both in terms of the variation in dose within objects such as animals and in terms of the probabilistic nature of energy deposition in small volumes like cells, will be discussed. The relevance of the stochastic nature of energy deposition to the biochemical processes that result in adverse effects will also be considered. Nature draws no clear-cut boundary between the physical, chemical, and biochemical stages of the interaction process. However, in this chapter we will attempt to deal with the physical processes, those that may depend on the atomic composition of the medium, but not on the active involvement of the chemistry or biochemistry of the target. Those processes that depend on the latter or on the molecular structure of the target will be dealt with in subsequent chapters. The processes covered in this chapter are generally completed quickly, probably in 10^{-13} seconds or less, while the chemical processes may extend over several seconds, and the biochemical ones may continue even longer.

After reviewing the current understanding of the physical processes relevant to the biological effects of irradiation, this chapter will attempt to outline the remaining gaps in our understanding and the additional capabilities which will be needed to properly understand the mechanisms of biological damage and to evaluate risks. This chapter will also briefly outline current research activities in these areas and recommend areas of basic and applied research where additional work will have the greatest impact on the reliability of risk estimates.

* The major part of the material in this report was prepared prior to June 1991.

INTERACTION OF RADIATION WITH MATTER

Ionizing radiations fall into two groups, directly ionizing and indirectly ionizing, on the basis of the types of interactions they undergo. The indirectly ionizing radiations, primarily photons (x and gamma rays) and neutrons, interact with atoms to produce energetic charged particles, such as electrons, protons, and heavier recoil nuclei. These secondaries as well as other charged particles, such as the beta and alpha particles emitted by radionuclides, are considered to be directly ionizing because they interact directly with the electrons of the atoms they pass. In general, the indirectly ionizing particles travel relatively long distances (up to tens of centimeters for energetic gamma rays) between interactions with the medium, but transfer a significant fraction of their energy in each interaction. Directly ionizing particles may interact more frequently, but often transfer only a small amount of their total kinetic energy to a secondary electron.

The interactions of indirectly ionizing radiations both with biological systems and the surrounding medium play a key role in the macroscopic distribution of dose. Neutrons interact with the nuclei of atoms through elastic, inelastic, nonelastic, and spallation processes (see Auxier et al. 1968; ICRU 1977). Since each of these processes produces charged particles and reduces the energy of the indirectly ionizing particle, many changes occur as a function of distance of penetration in the irradiated sample. Monoenergetic indirectly ionizing radiation is converted to a spectrum of directly and indirectly ionizing radiations in an exposed animal. Within that animal the energy of the secondaries, as well as the dose, varies in a complex way. However, it is the directly ionizing particles that produce most of the biological effect, so their interactions will be discussed in more detail.

The interactions of different directly ionizing radiations with individual atoms (such as in a gas) result in similar kinds of excitations and ionizations. It is primarily the distance between interactions which differs with different radiation qualities. However, in condensed phase materials, and particularly in macromolecules, the electric field of the moving particle may interact with the electronic structure of the molecule as a whole. This can result in differences in the energy transferred in solids and gases by a given type of particle and can also result in different types of interactions, depending on the charge and velocity of the incident particle. The differences in the distance between otherwise identical atomic products, as well as possible differences in the initial products, may result in differences in the effectiveness of the radiations. Spatial variations may result in different local concentrations of chemical products and may give rise to different products as a result of diffusion, recombination, and other interactions. These variations in initial radiation damage may also influence the nature and effectiveness of the biochemical processes acting on the damage.

When a charged particle (a positive ion or an electron) passes an atom, there is a Coulomb interaction with the electrons of the atom. The average energy lost by the particle is easily calculated, making it possible to accurately calculate the stopping power (ICRU 1978b; ICRU 1984; Bichsel 1968). However, each interaction is independent and can result in any amount of energy transfer up to the kinematic maximum for the interacting particles. The probability of energy transfer and its amount depend on the charge and velocity of the particle, the mass and electronic structure of the target, and the spatial separation between them.

Approximately comparable proportions of the energy lost by a typical particle are expended in excitation and in ionization of the target atoms.

In some of the ionizing events, the resulting secondary electron is emitted with enough energy to form a distinct track of its own; this is then referred to as a delta ray. The initial energy and direction of these delta rays are determined by the characteristics of the collision, but the low mass of a secondary electron relative to that of an atom results in large angular deflections in subsequent collisions. Hence, as delta rays interact they are scattered away from their initial directions. The spatial pattern of these various interactions serves to define the "track" structure of the charged particle.

The random nature of ionization along the track results in occasional short sections with unusually high or low ionization. Clusters of ionizations are also produced toward the ends of charged particle and delta ray tracks. Although it is reasonable to assume that regions of unusually high ionization density may contribute to the production of specific radiation products (Mozumder and Magee 1966) and there is evidence that the ends of electron tracks are highly damaging (Goodhead et al. 1978; Cornforth et al. 1989), there is no clear indication concerning the energy density required or the size of the region in which it must occur in order to produce a specific type of biological damage.

Efforts to understand the mechanisms of the effect of radiations of different qualities must begin with an understanding of the physical differences which characterize those radiations. It is not generally possible to measure the position of each ionization or excitation along a charged particle track. Measurement techniques with sufficient resolution and sensitivity have not yet been developed. As a result, reliance is placed on calculations of the relevant characteristics of tracks of charged particles to provide input for theoretical and experimental studies of the production of biologically relevant damage and of the way that damage is processed by the cell.

In models that describe effects in cells, it is sometimes convenient to utilize the track core and penumbra approximation (Chatterjee and Schaefer 1976) to estimate the effect of a fast charged particle. However, there is no clear distinction between these regions, but rather a continuous change in the average energy deposition as a function of distance from the path of the primary particle (Katz et al. 1972). More realistic impressions of the nature of charged particle tracks can be obtained from a Monte Carlo simulation (Paretzke 1987), and these calculations illustrate the limitations of attempts to describe realistic tracks in terms of simple average quantities.

Calculations of track structure, particularly the Monte Carlo simulations which give the greatest detail, require a knowledge of the cross sections for the relevant types of interactions and information on the energy and angular distribution of any products of the interactions that can cause additional ionization or excitation. Although many of the processes are not fully understood, theoretical predictions can be used to extrapolate between measured cross sections for single ionizations of gaseous targets by fully stripped charged particles. The resulting values are generally in good agreement with experimental measurements where they have been made (Manson et al. 1975), and they are used in most Monte Carlo calculations. However, satisfactory methods of predicting multiple ionization and of calculating the ionization due to partially stripped particles, thermalization of subexcitation electrons, and other processes are not available. The calculations currently utilize approximations which will require extensive testing

before they can be applied with confidence. The experimental measurement of multiple ionization cross sections has begun (DuBois 1984), but the other processes remain to be investigated. It has been shown, however, that the tracks of fully stripped protons and other common charged particles passing through a gas, such as water vapor, can be simulated with good accuracy (Paretzke 1987).

Monte Carlo simulations are also being undertaken for particle tracks in liquid water and for the chemical interactions of the initial ions and radicals with each other and the surrounding medium (Wright et al. 1983; Paretzke et al. 1986). However, the uncertainties in the input data are greater, and it is more difficult to test results for condensed phase media than for gaseous systems.

Within the limitations imposed by the availability of the physical data and the requisite computer time for Monte Carlo simulations, the positions of each ionization and excitation can be calculated for typical particle tracks. Similar calculations can be made for electron tracks resulting from the absorption of high-energy photons. However, these calculations result in the coordinates of very large numbers of interactions, for example, approximately 3000 ionizations and many times more excitations for a single 100 keV proton track. Because these are simulations (the actual positions of ionizations in a real cell cannot be calculated) and because each track will differ in detail, but will have a statistical similarity to other tracks with the same initial properties, it is evident that some means of characterizing tracks in a way that is relevant to their effectiveness in biological systems is needed. The concept of Linear Energy Transfer (LET) is an attempt to do this (ICRU 1970).

The unrestricted LET is the average rate of energy loss, or energy loss per unit distance, by the primary charged particle. The restricted LET, L_{Δ} , is the energy deposited close to the path of a primary particle. LET has proved to be of limited usefulness for two reasons. It is a measure of the energy lost by the primary particle, while the effect depends on the energy absorbed by the irradiated molecule. Furthermore, LET is the average loss rate, while the effect may depend upon the actual energy transferred to a small region. For example, endpoints such as mutation and transformation depend almost certainly on the damage in a volume no larger than a cell and probably in a much smaller volume, such as a single gene.

Relatively simple estimates of the transport of energy by delta rays, energy loss straggling, and charged particle range have been used to determine the range of sphere diameters over which the LET can be used to give a reasonable estimate of the energy deposited in the sphere (Kellerer and Chmelevsky 1975a). The strict application of this analysis is based on the assumption that the energy deposited in some specific volume, independent of how it is distributed within that volume, determines the biological effect; this is an unlikely assumption. However, the analysis shows that, at best, LET is applicable if the sensitive volume is within a relatively narrow range of sizes. If the sensitive volume is smaller, the energy transported outside the site by delta rays and energy loss straggling results in large deviations from the LET. If the sensitive volume happens to be larger than the range of the primary charged particles, the use of LET alone results in overestimates of the amount of energy deposited. Another consequence of the definition of LET is that the same charged particle species at widely different particle velocities can result in the same value of the LET (e.g., alpha particles at 13 keV and

12 MeV both have an LET of 50 keV/ μ m). However, these radiations have different track structures and may produce quite different biological effects.

An attempt to overcome the limitations of LET has been made by introducing the concept of lineal energy, y , defined as the energy deposited by a single track in a small volume divided by the mean isotropic chord length of that volume (ICRU 1983). Lineal energy and specific energy, z , the energy deposited divided by the mass of the volume, provide more information than the corresponding average quantities, LET and dose, because their distributions, as well as their means and variance, can be compared for different situations. Furthermore, lineal energy is a measurable quantity, and several techniques have been developed for measuring the distributions of y or their means (ICRU 1983). However, application of the concept of lineal energy requires choosing the diameter of the site in which it is to be specified. There is no assurance that a spherical volume of some particular size is the target for the production of biologically relevant damage. As a result, it has become common to utilize site diameters which are convenient for experimental measurements, usually in the vicinity of one micrometer. However, the importance of much smaller subcellular target regions has often been emphasized, and specific site diameter and even the shape of the volume are not inherent in the definitions of y and z . Monte Carlo track simulation can be used to calculate these quantities for any desired target. Spherical volumes down to nanometer diameters (Wilson and Paretzke 1981) and cylindrical volumes down to nanometer dimensions, including simulated segments of DNA molecules (Charlton et al. 1985), have been used.

The assumption that the probability of an effect is constant inside the volume and is zero outside the volume is inherent in the application of lineal energy. Alternative approaches, such as the proximity function that gives the distribution of distances between all pairs of ionizations or excitations along a track (Kellerer and Chmelevsky 1975b), have been suggested, but their applications rely on restrictive assumptions of the nature of the biologically relevant interactions. Because good data are lacking to test these assumptions or a mechanistic theory of the nature of these interactions, little use has been made of them.

Although it is unlikely that specific energy in any sharply bounded site is directly related to the mechanisms of the biological effect, several important features of the interaction of radiation with matter are easily discussed using this microdosimetric quantity. One of these is that specific energy, the microdosimetric analog of dose, for sites that have received an energy deposition depends very strongly on the size of the site. The reason for this dependence is that the amount of energy deposited is approximately proportional to the chord length of the charged particle track in the site, that is, linearly related to its diameter, while the mass of the site is proportional to the cube of the diameter. Furthermore, as the mean chord length decreases, the number of interactions between the charged particle and the atoms in the site decreases, with the effect that the relative variance in the energy deposited increases. The result is that the distributions of y and z become very broad and skewed toward large events. Thus, it may be misleading to think in terms of the average energy deposited (dose) to very small structures such as chromatin. If energy deposition occurs, the energy per unit mass is likely to be very large, but most segments of chromatin receive no deposition.

Because the energy deposited along the track of a charged particle is usually large, after a small dose the average distance between charged particle tracks may be considerably larger

than the structures in which relevant damage occurs. In such a situation, the specific energy in the target depends on the characteristics of the charged particle track and on the mass of the target, but not on the dose to the object containing that target. For example, taking $7\mu\text{m}$ to be the diameter of a cell nucleus, the mean specific energy in single events due to 1.0 MeV neutron irradiation is approximately 0.14 Gy, while it is 0.001 Gy for ^{60}Co irradiation. This disparity reflects primarily the difference in the stopping power of the proton and electron secondaries of these radiations.

The passages of charged particle tracks through small volumes, such as nuclei of cells, are statistically independent events, and the number of such events follows a Poisson distribution. The average dose for all the nuclei in a sample, including those which do not receive an energy deposition, is the mean specific energy deposited in an interaction times the average number of interactions per nucleus. Thus, for a dose of 0.01 Gy (1.0 rad), nuclei exposed to ^{60}Co irradiation receive an average of 10 events per nucleus, but the nuclei exposed to 1.0 MeV neutrons each receive an average of only 0.07 events per nucleus. Hence, in the latter situation one in 14 nuclei receives an energy deposition event.

At the upper end of the range of doses of interest in radiation protection, low-LET radiations produce several energy deposition events in almost every nucleus that is exposed, and the amount of energy deposited per nucleus is relatively constant. For similar doses of a high-LET radiation, the majority of the nuclei do not receive energy depositions, but those which do generally receive a relatively very large amount. At these doses the effects of a high- and low-LET radiation evolve from quite different physical patterns of energy deposition at the cellular level. Cells or nuclei of a tissue exposed to a low-LET radiation all receive similar amounts of initial damage. In contrast, a small fraction of the cells (or nuclei) in a sample exposed to the same dose of a high-LET radiation receive a much larger specific energy, although the majority of the cells receive no damage at all. Furthermore, if the radiation is delivered at a low dose rate, the individual events registered in cells (or nuclei) irradiated with a low-LET radiation will be well separated in time, and the effects of one event may be modified or eliminated by biological processes before additional events occur.

Electron tracks end with a short region of high-density energy deposition, which is capable of producing some of the same products as high-LET radiations, but these regions contain only a part of the total energy deposited by most electrons, and they are widely separated. High-LET radiations produce a high concentration of damage along tracks that may cross a cell nucleus, and this may cause differences in the biological processing of the damage. At extremely low doses the probability of two or more tracks crossing one target becomes quite small and, consequently, multievent effects become less probable. In addition, for a small dose, two or more events separated in time will occur less frequently in a small target, thus minimizing the likelihood of a dependence on the dose rate. It is noteworthy, however, that although the secondaries produced by neutrons have high-LET values, the absorption of neutrons by atoms results in the emission of x rays which, in a given target, may deposit energy separated in time and space from the passage of the high-LET particle.

In the foregoing example, reference was made to spherical targets the size of a cell nucleus. Similar considerations apply to other small sites, such as specific regions of chromatin.

The numerical values for the specific energy for single events will shift roughly in proportion to the mean chord length in the site divided by the volume of the site.

DOSIMETRY

The average energy imparted per unit mass at a point is a convenient way to specify the amount of radiation delivered. This quantity, the absorbed dose, can also be defined as the limit of the mean of the specific energy as the mass of the site approaches zero (ICRU 1980). Alternatively, the amount of radiation can be specified in terms of fluence of primary or secondary particles. In addition, since equal doses (or equal fluence) of different radiations result in different effects, it is necessary to specify the "quality" of the radiation. Unfortunately, as discussed in the previous section, no universally acceptable way of specifying radiation quality has been identified. In the absence of a good way of describing radiation quality, the spectrum of charged particles delivering the energy, in terms of mass, charge, and velocity, can be used to characterize the radiation. This procedure provides a full description of the radiation field which should facilitate future analysis as new methods of interpretation become possible.

The absorbed dose (or the fluence) and the charged particle spectrum are needed at the points where biologically relevant damage occurs. However, these characteristics can change dramatically over short distances, especially near discontinuities in the density or atomic composition of the target material. In some experimental situations, notably the irradiation of very thin samples with a charged particle beam from an accelerator, the radiation characteristics at the target are very nearly the same as that of the incident beam, and any differences are relatively easy to calculate. Such arrangements are useful in studying the mechanisms involved in radiation effects at the cellular level because they enable a specific characterization of the radiation interactions.

However, in most situations, in radiation protection as well as in experimental investigations, the incident radiation is indirectly ionizing. As neutrons and photons interact with the absorber, part of their energy is transferred to ionizing particles in discrete interactions. A buildup of the fluence of charged particles and dose occurs just inside the absorber. The rate of buildup and the difference between surface dose and maximum dose depends on the ranges of the secondaries. Furthermore, as the primaries lose energy their mean free path and the energy they transfer to secondaries change. The result is not only a change in dose with depth, but also a change in the "quality" of the secondary radiation. Such changes are often difficult to calculate or to measure, but can be biologically significant.

Most radiation sources, especially neutron sources, do not produce monoenergetic beams, and the physical surroundings in which most objects are irradiated lead to a variety of scattered particles and secondary radiations. This complex spectrum of incident and scattered radiations further complicates the problem of determining the actual dose and radiation quality at the relevant points within the target. In this respect, neutrons are particularly troublesome. Most neutron sources also produce photons of various energies, and when neutrons stop in hydrogenous material they are usually captured by a proton or carbon atom and result in a high-energy gamma ray. Thus, neutron irradiation inevitably involves a mixture of radiations with

both high- and low-LET charged particle secondaries. Furthermore, neutrons will backscatter from the air in an irradiation room, as well as from the walls and other parts of the experimental setup. The scattered neutrons have lower energy and, thus, may have different biological effectiveness than the primary radiation.

Appropriately designed irradiations, careful dosimetry, and appropriate analysis are necessary to ensure that the physical characteristics of a neutron exposure are understood so that the biological consequences of the irradiation can be properly interpreted. During the past decade appreciable progress has been made in the design of exposure arrangements and the performance of dosimetry for biomedical applications. Special precautions, sometimes including multilateral irradiation, must be taken to ensure a homogeneous distribution of the absorbed dose over the exposure field (ICRU 1979; Zoetelief et al. 1985). The relative contribution from photons will increase with the size of the biological specimen or when a multiplicity of objects is irradiated. In view of the differences in the biological effectiveness of photons and neutrons, it is important to determine and specify their respective contributions to a given endpoint, as well as their possible combined effects in the instances of low-dose, low-dose-rate exposures.

When a series of experiments is performed at a variety of dose rates, it may be unavoidable that different exposure arrangements are applied. In such a situation, the consistency of the dosimetry for total absorbed dose and the portion attributable to photons should be checked. A number of photon and neutron dosimetry intercomparisons performed during the past 15 years initially showed appreciable differences, in some cases in excess of $\pm 20\%$ from the average or reference value, but the differences can be greatly reduced by careful practice. For uncontrolled exposures in mixed neutron/photon fields, larger variations in the absorbed dose may be expected.

Dosimetry intercomparisons have been of value in assessing the state of the art, in providing a check on techniques for an individual dosimetrist, and in stimulating the development of improved instrumentation. The participants in the International Neutron Dosimetry Intercomparison, INDI, (ICRU 1978a) and the European Neutron Dosimetry Intercomparison Project, ENDIP, (Broerse et al. 1978a; ICRU 1989) generally employed tissue equivalent ionization chambers to determine the total absorbed dose and ionization chambers of low sensitivity to neutrons (C-CO₂ or Al-Ar chambers) or a Geiger-Muller counter to derive the contribution from photons. Various types of tissue equivalent ionization chambers have been characterized (Broerse 1980), and neutron dosimetry protocols have been drafted separately for the United States (AAPM 1980) and Europe (Broerse et al. 1981). These protocols are intended to assure consistency in dosimetry procedures and provide recommendations for basic physical data. In a second European Neutron Dosimetry Intercomparison Project, ENDIP-2, the European neutron dosimetry protocol was adopted and a common set of instruments was introduced at the institutions involved. In general, agreement within $\pm 2\%$ for the total absorbed dose was obtained between the measurements of the local dosimetrist and those of the visiting team (Zoetelief and Schraube 1985).

In Europe a number of institutes involved in studies of late effects in experimental animals have joined their efforts by establishing the European Late Effects Project Group (EULEP). An exchange of biological results will be useful only when a common methodology is established for pathology and dosimetry. Within the framework of EULEP, five consecutive

x-ray dosimetry intercomparisons have been performed, and a dosimetry protocol has been amended (Zoetelief et al. 1985). These activities have resulted in improved consistency of x-ray dosimetry at the 14 participating institutes.

Perturbations in the equilibria of secondary charged particles and consequent perturbations of the absorbed dose are also important items to be considered. Appreciable variations in absorbed dose will occur at the interface of materials of different composition. For example, when cultured cells attached to the bottom of a culture flask were irradiated with 15 MeV neutrons through the medium, an increase of 17% in absorbed dose was observed relative to irradiation through the polyethylene bottom of the flask (Broerse and Zoetelief 1978). It can be expected that larger differences will occur for lower energy neutrons.

For the irradiation of larger objects, the reference points for the stated dose levels should be identified. Reports about LD₅₀ values in man are not always comparable since values of the dose can refer to the reading of a personal dosimeter worn on the chest, the midline absorbed dose, or the average absorbed dose in the bone marrow (Carmichael 1988).

The problems outlined above are particularly important when interspecies comparisons are being made, and, particularly, if the organisms being compared differ greatly in size. Thus, results of exposure of cells, mice, and large mammals to identical beams of neutrons cannot be directly compared because of changes in the quality of radiation as neutrons penetrate tissue. The larger organism will receive, in the center of its body, radiation with a large low-LET component and a degraded neutron spectrum, as compared with either cells or mice. Similar, but less severe, problems occur in comparing results with cells and mice.

Each experiment must be evaluated carefully to ensure that the best possible data result from the measurements. Often, experiments which were originally designed to produce one type of observation can yield interesting and potentially valuable observations in another area. In such situations, seldom is the type and amount of dosimetry data available which would have been required if the collateral effect had been anticipated. Occasionally, retrospective studies can produce the missing physical data, but often there is insufficient detail in the records of the exposure, thus precluding a full interpretation of the biological observations. For this reason, and because present dosimetry techniques may involve significant errors, it is recommended that in addition to absorbed dose and radiation quality at the point of interest, a detailed description of the radiation source, incident radiation, target configuration, and the environment should be given for all neutron experiments.

RESEARCH NEEDS

An examination of what is understood about the interaction of radiation with biological material, the methods for describing that interaction, and the means of making the appropriate measurements reveals a number of areas where additional information is needed. Some of these remaining problems are of a very basic nature or require more or better data on both the basic physical and chemical processes. However, many other problems require the development of improved dosimetry techniques or instrumentation and will be dealt with separately. The precision and accuracy required in the dosimetry depend on the biological effect being measured,

but uncertainties in the physical measurements generally should not be allowed to contribute significantly to the variance in the measurement of the biological effect. As biological systems are refined, the variance attributable to the biological system tends to decrease and more accurate dosimetry becomes justified. A difficult but realistic goal would be to develop the ability to make neutron dosimetry measurements with an accuracy of $\pm 5\%$ when required, as well as to adequately characterize the quality of the beam at the points of interest.

Basic Research

The measurement techniques and underlying physical data necessary to evaluate photon and neutron doses have been accumulated over many years. Although the cross sections, attenuation coefficients, kerma factors, and so on are well known for common radiations over the prevalent energy range, there are regions where these factors are not adequately known. Additional data are needed particularly for high energy (above 14 MeV) neutron cross sections. In addition to data needed for neutron transport calculations, emphasis must be given to the high-LET products of nonelastic and spallation reactions since they may have a dramatic impact on the effectiveness of the radiation. In fact, in order to achieve the accuracy required for the dosimetry of biological effects experiments and the specification of radiation quality, many cross sections may have to be determined with added accuracy. These measurements require the availability of suitable monoenergetic neutron sources.

The gas-phase doubly-differential cross sections for ionization, the basic input for the simulation of charged particle tracks for common ions that are fully stripped of electrons, are known with reasonable precision. However, the cross sections for infrequent reactions, such as multiple ionization, have not yet been adequately investigated. Similarly, the effects of screening of nuclear charge by the electrons of partially stripped particles, and the contribution of other sources of delta rays, such as those produced by charge exchange processes, are not yet known. Experimental measurements are needed, especially because a substantial component of the dose from fission neutrons, which are of a particular practical relevance, is deposited by recoil protons of energies sufficiently low (< 0.3 MeV) that they may not be fully stripped and, therefore, cannot be accurately simulated by current track structure Monte Carlo codes.

The potential relevance of some of these processes to the overall track structure and the potential biological effectiveness should be investigated using sensitivity analysis in the modeling process. However, due to the lack of specific knowledge about the biological significance of patterns of energy deposition on the nanometer scale, the evaluation of the relevance of particular energy transfer processes can only be estimated, and this should be done conservatively. The measurement of ionization may be a satisfactory approximation of energy deposition in many experimental situations, but it is limited when consequences in small sites or macromolecules with complex structures are considered.

Although biological systems exist only in the condensed phase, most of the data used in calculating detailed track structures and most of the experimental measurements of energy deposition on a microscopic scale are for gases. This state of affairs exists because the close proximity of additional atoms and molecules in a solid or liquid interferes with the measurement

of the secondaries of individual interactions. However, this close proximity can also result in the participation of adjacent atoms in the interactions. This interaction is known to alter energy transfer characteristics and possibly some important features of track structure. Optical data, such as oscillator strengths, can be used to help determine the magnitude of this phase effect and to correct for it, but additional theory is needed to determine the effects of phase on all of the energy deposition processes and on the types of chemical products resulting from different radiations. Furthermore, additional experimental techniques are needed to test the results of these theoretical developments. The measurements of electron emission from small clusters or thin films of atoms or molecules might be useful.

The processes following the initial ionizations and excitations also require additional study in realistic systems. The diffusion and interaction characteristics of ions and radicals in dilute solutions are reasonably well known and are being used to calculate, as a function of the time after irradiation, the distribution of those chemical species that are biologically relevant (Wright et al. 1983). However, very little is known about these processes in the structured, heterogeneous systems characteristic of protoplasm. In addition, the ordered nature of the proteins and water molecules surrounding DNA and the tertiary and higher order structure of chromatin (Ljungman 1991; Chiu et al. 1992) may influence the interactions of radicals with chromatin and, thus, the subsequent effects of those interactions. Radiation also causes direct ionizations and excitations in the DNA and in associated proteins, and localized clusters of such damage may have direct biochemical consequences with low-LET and especially high-LET radiations (Ward et al. 1985; Ito 1987; Goodhead 1987).

An underlying need is a way of describing the processes of energy deposition that provides an adequate correlation with the biological effects produced. This need is not simply a problem of radiation physics, but it must also involve many advances in radiation biology and chemistry. The actual mechanisms involved, when understood, will undoubtedly preclude a simple one- or two-parameter description of the quality of the radiation. Practical requirements may result in choosing a less than optimum description for particular applications, but the better we understand the physical processes the better we will be able to devise a workable compromise.

Applied Research

A number of improvements in instrumentation and the techniques for their use are needed not only to improve the quality of the dosimetry used in experimental studies of the effects of different radiations, but also to improve the quality of operational health physics data. Improvements are important because such measurements and the records to which they will give rise will have to be used for the study of dose-effect relationships among occupationally exposed individuals. The option of utilizing the maximum quality factor for all neutrons, if their LET distribution is not known, provides conservative radiation protection, but it seriously distorts the record with respect to actual doses. There is the risk that at some future time these overestimates of dose may be applied to observations of rates of specific effects, and this may result in underestimates of risk per unit dose.

Most instrumentation for neutron dosimetry, particularly in the practice of radiation protection, but also in some experimental situations, does not actually measure the dose. Instead, some other physical quantity is measured which is related to dose and the neutron spectrum. This quantity may also have an energy-dependent response to other radiations. For example, many area monitoring devices detect thermal neutrons and rely on a moderator of a specific size and shape to alter the incident spectrum of neutrons so that the number of thermals reaching the sensor is approximately proportional to the dose equivalent. Similarly, albedo dosimeters (TLD personnel dosimeters) detect neutrons scattered back to the detector by the body of the wearer. Again, the response is highly energy dependent. In these and similar situations, the normal practice is to calibrate the device in a radiation field that approximates the spectrum which will be encountered in use. However, if the spectrum of neutrons changes, the calibration is no longer valid. Thus, some form of instrumentation for monitoring neutron quality (microdosimetric or other) which can be used to monitor radiation sources periodically and can be operated in a few minutes by a nonexpert operator is needed to detect inadvertent changes in the radiation quality.

Significant new developments are needed in neutron health physics. Improved dosimeters (passive indicators of total dose equivalent) with uniform energy response and a reliable indicator of radiation quality are needed. Similarly, area monitors (active devices indicating dose equivalent rate) whose response is accurate over the entire range of neutron energy and in mixed neutron plus photon fields are required. Techniques to determine dose to specific organs, probably involving the use of multiple dosimeters and computer analysis to estimate the dose at various depths, will also be necessary. Small detectors, suitable for measuring dose and spectrum inside a phantom, are needed to verify organ dose calculations. Improved "biological dosimeters", measurements of specific changes in individuals that can be correlated with radiation exposure, will be needed to determine doses received when conventional dosimeters were not used.

Improvements are needed in the instruments and techniques for measuring and calibrating irradiation facilities. Improved neutron spectroscopy, that provides the opportunity to measure the field without seriously distorting it and provides consistent results between different measuring systems, is needed. Improved gamma-ray dosimeters, with low or very predictable neutron responses, that accurately measure the photon dose in unknown neutron fields, are needed. Systems for measuring lineal energy or related quantities for site sizes below $0.5 \mu\text{m}$ should be developed.

It is likely that risk estimates for neutrons and other radiations will continue to depend partly on the evaluation of epidemiological data and on the unexpected results of experiments designed to investigate particular endpoints. These data rely on retrospective evaluation of the dose, and improved testing of the calculations involved is required to provide the needed accuracy.

Experiments to determine biological responses to neutrons require improved neutron sources, as well as more precise dosimetry. Most of the data now available have been obtained with fission spectrum neutron sources. These are generally preferable to higher energy broad-spectrum sources (such as those used in radiotherapy) because the latter produce a wide spectrum of both high- and low-LET secondary particles. The effects of these particles are not

readily open to separate analysis. Although fission neutrons are relevant to most anticipated sources of accidental or occupational neutron exposure, they are not good sources of data for the analytical evaluation of the effect of neutrons as a function of energy or other indicators of radiation quality. For these purposes, relatively intense sources of neutrons of different energies, each with a narrow energy spread, would be preferable. The use of the narrow range of energies places a clear upper limit to the energy distribution of recoil particles and results in a relatively simple distribution of recoil particles. It also simplifies the problem of determining the dose and the spectrum of neutrons as a function of depth in the animal. If there are interactions between different biological products produced by the different types of secondary radiation, then experiments with each type of radiation controlled independently will be needed to unfold the response of the system.

Chapter V

CHEMICAL AND MOLECULAR MECHANISMS UNDERLYING CELLULAR RESPONSES*

INTRODUCTION

The challenge for radiation chemistry and biochemistry, with respect to the assessment of the biological effectiveness of neutrons and other radiations, is to understand how the initial radiation-induced events are converted into biological damage in cells and tissues. The physicochemical changes which follow the passage of an ionizing particle through cells result in molecular changes which, in turn, initiate a sequence of biological reactions that may lead eventually to an observable biological endpoint.

A wide range of molecular and enzymatic reactions occur in cells. Some protect the cell from damage; others participate in the repair or fixation of the damage. The identification of these processes and the physiological ramifications accompanying them should lead to an understanding of the importance of specific lesions in giving rise to radiation effects.

CURRENT STATUS AND GENERAL CONSIDERATIONS

Energy Deposition and Its Immediate Consequences

Radiation interacts with matter, including the living matter of cells, by depositing energy in molecules. Some molecules are raised to excited states and others are ionized. Excited molecules may lose energy by light emission, they may dissociate to form pairs of free radicals (molecular fragments that have unpaired electrons), or they may undergo various other processes. In the excited state, the conformation of molecules is different from that in the ground state. When excited, a molecule begins to change its conformation within 10^{-14} seconds. This aberrant conformation is maintained until the molecule returns to the ground state, in 10^{-8} seconds or more, that is, a long time relative to the times of molecular vibrations and collision frequencies. If the excited molecule forms a pair of free radicals, the conformation of each fragment is different from that of the undamaged molecule. The radicals in a given pair may recombine with the release of energy, but the probability is large that they will diffuse away from each other. Because the chemical reactivity of free radicals is high, they generally react with neighboring molecules on a time scale of milliseconds or less.

Molecules that have become ionized, that is, have lost an electron, form radical cations that undergo conformation changes similar to those described for excited molecules. In addition,

* The major part of the material in this report was prepared prior to June 1991.

they may recapture the lost electron and become excited molecules, or they may lose a proton and become neutral radicals. These radicals often are different from those formed by excited molecules. The radical cation may react with other neighboring radicals, but in aqueous media this is an unlikely reaction because deprotonation is usually a very fast reaction. The electron, if not recaptured by its parent radical cation, may diffuse several molecular diameters and fall into a potential-energy trap in water to become a hydrated electron. In this form, it is highly reactive and can be captured by many kinds of molecules to form a radical anion. Electrons may be captured before they are hydrated, also producing radical anions.

In pure water, it is believed that about six new chemical species are formed in the physicochemical stage following irradiation and that these can take part in at least 25 different chemical reactions during the subsequent chemical-reaction phase (Magee and Chatterjee 1987). These changes occur in a millisecond or less after each act of energy deposition.

Radiation Track Structure and the Spatial Distribution of Chemical Species

The nature and kinetics of the chemical changes induced by radiation are strongly governed by the fact that the initial energy deposition and, consequently, the first identifiable chemical species are concentrated in structured tracks. Subsequent early chemical reactions are accordingly highly nonhomogeneous. In a common simplified approach, the spatial features of low-LET track structure are described as small isolated clusters of ionizations (called spurs) up to about 100 eV average energy. This accounts for about 70% of the energy deposited by low-LET radiations, but ignores the remaining 30% which is deposited mostly in more dense highly-localized energy bundles at the terminal track-ends of the electrons. In high-LET radiations, including fission neutrons, the clusters are closer together and may be much larger due to closely adjacent primary ionizations, overlapping spurs, and electron track-ends along the paths of the recoil protons. Some models of radiation chemistry have considered such features in terms of average tracks and limited diffusion of initial chemical products (Chatterjee and Magee 1980) or have included random features of the tracks and the reactive species generated in them by the use of Monte Carlo methods (see Chapter IV; Wright et al. 1985).

Radiation Chemistry in Elementary Systems

Radiation chemistry has been extensively investigated with low-LET radiations and elementary model systems: dilute aqueous solutions; frozen matrices or glasses made of concentrated alkali, salt, or acid; and compounds in the solid state. Experiments in the latter two systems frequently have been carried out at temperatures as low as that of liquid helium to decrease the rates of radical reactions. Thus, it is necessary to use great care in applying results obtained in these systems directly to living cells.

Nonetheless, investigations as above have been useful because they have indicated what processes can occur in irradiated systems and they have suggested what kinds of reactions should be looked for in biological systems. To summarize, the low-LET radiolysis of water yields hydroxyl free radicals ($\text{OH}\cdot$) and hydrated electrons (e_{aq}^-) in concentrations of about 2.7×10^{-9} moles per dm^3 per cGy, hydrogen peroxide (H_2O_2) and hydrogen (H_2) in concentrations about a fifth as large, and a small yield of hydrogen atoms ($\text{H}\cdot$). High-LET radiations give lower yields of radical products and higher yields of molecular products.

The hydroxyl free radical ($\text{OH}\cdot$) is one of the strongest and most reactive oxidizing agents known. It will react with nearly all organic molecules in a cell, frequently on the first collision. The hydrated electron likewise is highly reactive. It reacts as a strong reducing agent with many compounds. The usual reaction is to add to a molecule to form a radical anion. The latter may dissociate or transfer the electron to another molecule to form a thermodynamically more stable radical anion. Ultimately, all radicals recombine to form entities that do not have unpaired electrons.

A common reaction of electrons in systems containing oxygen is to add to the oxygen to give the superoxide radical anion (O_2^-). This radical and oxygen itself may play dominant roles in radiolysis in biological material.

Experiments in aqueous model systems have shown that DNA reacts rapidly with $\text{OH}\cdot$ producing changes in DNA conformation, base damage, single and double strand breaks, and cross-links with other DNA molecules, proteins, and other organic molecules. Hydrated electrons also react rapidly with DNA, but they do not seem to damage it, possibly because the electron is transferred rapidly to other molecules.

The deposition of energy by radiation directly in DNA produces excited and ionized molecules as described previously. Low temperature experiments suggest that the earliest postirradiation DNA products are a radical cation with the charge localized on a guanine moiety and a radical anion with the charge on a thymine or cytosine moiety. Radiolysis of proteins produces analogous changes. Lipids are readily oxidized in aerated solutions with major changes in conformation at the double bonds of unsaturated hydrocarbon chains.

The chemistry of cellular constituents is complicated by the fact that the biological material itself is highly structured, concentrated, and reactive. Very large numbers of possible reactions and products can exist following irradiation, yet it is likely that relatively few of them have final biological consequences for the irradiated cell. Ionizing radiation typically produces on the order of 10^3 ionizations per cGy in a mammalian cell nucleus $9 \mu\text{m}$ in diameter, with about ten of these ionizations being in or close to the DNA itself. These ionizations can initiate reactions within or between any of the molecules in the nucleus.

All of the chemical products described above result in changes in molecular conformations. In most cases, the chemical nature and the properties of the products are significantly different from those of the original molecule, and what is left of the original molecule may no longer be able to perform its function. Whether this result affects a cell depends on whether the damaged molecule can be repaired, replaced, bypassed, etc., and whether its function is vital.

Primary Cellular Targets

Because of the random nature of the deposition of the energy of ionizing radiation in living tissue, it may be possible for damage to be localized in any of the myriad of molecules and structures which make up cells. However, the high redundancy of the majority of molecular constituents and the ease with which they can be replaced even in lethally irradiated cells suggests that they are not the sites of critical damage. It is more likely that the critical molecular species are those of minimal multiplicity, with large biological amplification of the damage, that have maximum impact on the future function of the cell, that is, the cellular genome. Considerable evidence indicates that a primary molecular target of ionizing radiation-induced cellular change is DNA, or, more specifically, nuclear DNA and its molecular environment, as in chromatin. Damage to DNA can lead to cell death, mutagenesis, carcinogenesis, and aberrant development. Damage to other cellular targets, such as membranes, may result in other responses, for example, disruption of lysosomal, Golgi, or plasma membranes; activation of membrane-localized second messenger pathways; specific gene induction; apoptosis; or defective spindle formation.

Damage to DNA

Damage to DNA has been the subject of considerable research for more than 30 years. Most of the information available concerns low-LET radiation. A great deal of information has been assembled (see reviews by, e.g., Wallace 1983; Hutchinson 1985; Teoule 1987). DNA lesions which have been identified include single and double strand scissions, chemically modified DNA bases and sugars, and DNA-to-protein and DNA-to-DNA covalent cross-links. Because of the large array of lesions, it has not been possible to identify unequivocally a single type of lesion as the unique causative agent responsible for any of the acute or late effects of high- or low-LET radiation. Although there is evidence for the importance of double strand breaks in cellular lethality, most double strand breaks are repairable and, therefore, without observable effect. It may be that under the proper conditions other lesions can produce observed deleterious physiological responses.

Efficiency of Radiation and Dose/Damage Relationships

Compared to other agents, ionizing radiation is highly efficient in cell killing when efficiency is expressed as the number of DNA lesions per lethal hit (Elkind and Redpath 1977; Ward et al. 1987). Fewer DNA lesions are produced at a particular level of cell killing by ionizing radiation than are produced by other agents (e.g., ultraviolet radiation and chemical mutagens/carcinogens). As a consequence of the efficiency of radiation in initiating deleterious cellular responses and the insensitivity of most available assays in the detection of radiation-induced DNA lesions, the study of the production and repair of lesions in DNA often requires

the exposure of cells to doses of radiation which are large relative to those which are biologically relevant, for example, doses which are larger than those corresponding to 10% survival. In terms of mutagenic and oncogenic endpoints, the relevant doses are usually considerably lower, and the doses of greatest concern in risk assessment even lower still, that is, doses which cause little or no cell killing. The greater lethal efficiency of neutrons makes this problem of relevant doses even more evident.

Because it has been found that most initial lesions in cellular DNA are produced linearly with dose, the use of large supralethal doses to generate a sufficient signal for accurate measurement by a particular assay is acceptable as an interim measure until more sensitive assays are developed. However, the measurement of the biochemical repair of DNA lesions after high doses of radiation should be conducted and interpreted with care. Some normal processing of lesions may be inhibited by high radiation doses, and catabolic processes may be initiated in DNA because of concomitant damage to nuclei, lysosomes, or other cellular membranes.

Limitations of Simplified Experimental Systems

A variety of simplified test systems has been used to investigate the effects of radiations on DNA. These range from free bases and nucleotides, to isolated plasmids or other DNAs in solution, to DNA-protein complexes of various constitutions, to isolated chromatin, and to intact cells. The choice of a particular system depends on the questions to be addressed and the acceptability of the limitations which are imposed. Aqueous solutions of DNA containing high concentrations of radical scavengers simulate the milieu of the cell by limiting the range of migration distances for radicals, whereas solutions of isolated chromatin retain the structural association of DNA with protein. Dry films of DNA permit the study of the direct effects of radiation. Ultimately, the radiobiological challenge is to understand the chemistry of damage to DNA and associated structures in cells. Thus, the data derived from simpler systems necessarily will be limited in usefulness for the following reasons.

(1) Cellular chromatin contains DNA, proteins, and RNA folded into specific and complex structures which have not been duplicated in a cell-free system. There is evidence that the environment created by the folding of chromatin influences the observed radiation responses (Chiu et al. 1982, 1986, 1992; Ljungman 1991).

(2) The extensive scavenging capacity of the intracellular milieu also is not well duplicated in cell-free preparations. Large differences may be expected in the yields of particular kinds of damage to biological macromolecules, such as DNA, when they are irradiated within intact cells as compared to irradiation in dilute solutions. Average migration distances of primary water radicals within cells are small, estimated to be 2-5 nm, because of high concentrations of endogenous reactive species and because the DNA is in very close association with bound water and protein. There is considerable uncertainty about the relative importance

of indirect radiation damage to DNA within cells due to reactive chemical species versus damage due to ionizations or excitations directly in the DNA. The relative balance depends on the nature of the DNA damage being considered and on the type of radiation.

Distribution of Damage in Sensitive Cellular Targets

The distribution of radiation-induced damage in DNA and in chromatin has not been extensively investigated. The following points need to be considered.

(1) Absorption processes result in clusters of ionizations. In this context, a cluster is defined as the multiple, coordinated ionizations that result from a simple localized deposition of energy and that occur within a small volume comparable to the size of the biological target (2 nm diameter for the DNA helix, 6 nm x 11 nm for a nucleosome). The energy involved ranges up to hundreds of eV per cluster for low-LET radiation and to much higher energies for high-LET radiation (Goodhead 1989). If a cluster is formed in the vicinity of DNA or in the DNA, the probability of more than one lesion occurring in a small region of the molecule is increased. The result may be sites with a number of closely spaced lesions. These sites have been referred to as "locally multiply damaged sites" by Ward (1987). It has been proposed that such sites are important because cellular repair might be inefficient in handling one lesion in the presence of another lesion in the immediate vicinity. In addition, some of the locally multiply damaged sites may be double strand breaks.

Simple single-radical products, such as DNA base changes, may be produced principally by indirect action. DNA double strand breaks, and even more complex products, may result in greater measure from direct action of the high ionization-density components of the radiation tracks. Such multiple ionization events are likely to be important contributors to cellular radiation effects even for low-LET radiations. They may be the major contributors to DNA damage from slow, densely-ionizing particles, such as the secondaries resulting from neutron irradiation. A clear distinction between the effects of direct and indirect action in DNA is blurred by the possibility that the final damage, resulting from a track which produces a cluster of ionizations, may be distributed between the DNA and closely adjacent molecules such as bound water, protein, and/or lipid.

Despite these uncertainties, it seems likely that relevant damage to biological macromolecules following low doses of fission neutrons will be due to highly localized clusters of ionizations and excitations either within, or very close to, the target molecules. These effects will be almost exclusively due to single tracks because, for the doses of interest, the mean distances between tracks will be orders of magnitude larger than the diameters of the tracks.

(2) Because DNA lesions are generally produced at or near the site of the production of radiation-induced radicals, the spatial distribution of DNA lesions should reflect the spatial distribution of the initial energy deposition. However, it has also been suggested that DNA radicals or excited states in the DNA may travel from their point of origin to other DNA

regions which may serve as energy sinks (e.g., Baverstock and Cundall 1988). Electron migration in protein and electron transfer to DNA are processes which have been observed (Cullis et al. 1987). Examination of the relevance of such processes will require the identification of those sequences or structures in DNA which serve as preferential sites for the ultimate deposition of DNA damage. However, biophysical analysis indicates that features of the energy distribution characteristic of the initial radiation track would be maintained during such migration.

(3) The standard structure of B-DNA as a right-handed cylindrical rod having base pairs approximately perpendicular to the helical axis is insufficient in several respects to explain the structure of cellular DNA. For example, sequences rich in G-C base pairs have a tendency under the proper conditions to be converted into a left-handed helix, Z-DNA (Rich et al. 1984). Sequences rich in A-T base pairs can form bends and kinks in the B-DNA chain (Wu and Crothers 1984; Trifonov 1985; Ulanovsky and Trifonov 1987).

In addition to structural variations resulting from the sequence of bases, other variations depend upon the interaction of DNA with protein. DNA is wrapped around successive octamers of histones to form nucleosomes. The further winding of nucleosomes generates "solenoids" and "superbeads" which form more complex and more compact structures in metaphase or in heterochromatin during interphase. During most of the cell's growth cycle, at intervals of 30-200 kb (kilo base pairs), chromosomes appear to be attached to an intranuclear protein scaffold or matrix, resulting in loops of DNA anchored to the matrix (Pienta and Coffey 1984; Gasser and Laemmli 1987). It has been proposed that the nuclear matrix is the site of DNA replication (Pardoll et al. 1980), transcription, and some types of DNA repair. The nuclear matrix is also the site of topoisomerase II, an enzyme essential for the conformational changes associated with DNA replication and which maintains chromosomal loops in a supercoiled state (Berrios et al. 1985). Nucleosomes tend to relieve the torsional strain of supercoiling, and some nucleosome-free supercoiled regions near actively transcribed genes may be preferential sites for attack by nucleases and other damaging agents because of the unrelieved strain on the DNA structure and its greater accessibility to the damaging agents (Elgin 1988). Complex conformational changes in chromatin are necessary for gene transcription (Felsenfeld 1992). Deciphering the role of these and other structural variations of the chromatin target in radiation damage is a major challenge in radiobiology, in general, and in understanding differences between high- and low-LET radiation action, in particular.

(4) Biophysical models of radiation damage have inferred from dose-response relationships that the sensitive targets for different endpoints may be much larger than single DNA base pairs or even most genes (Goodhead 1985b). Furthermore, the majority of measured DNA damage, that is, single strand breaks and nucleotide base alterations, is repaired and is therefore of no consequence. Repair of potentially lethal DNA lesions permits damaged cells to survive and increases the likelihood that low-frequency mutagenic events will be expressed.

(5) Mutations resulting from radiation damage may occur at the site of the initial DNA lesion or at a site remote from the initial lesion as a result of enzymatic processing of the primary damage. Ionizing radiation produces all types of mutations, with a high proportion being large-scale genomic rearrangements (see Chapter VI). The induction of such large-scale mutations may result from radiation damage at any point within a large structure or domain of DNA that is consistent with viability.

(6) The physiological status of the cell must also be considered. Factors such as metabolic state; position in the cell cycle; the presence of nutrients, hormones, and other growth factors; cell shape; and cell-to-cell contacts may affect the conformation of molecular targets, as well as the ability of the cell to process radiation damage.

RESEARCH NEEDS

Emphasis on Fundamental Research

To have long-term value, the proposed research should be carried out at a fundamental level with the aim of learning more about mechanisms in radiation chemistry and biology. Such information will provide the conceptual framework for evaluating animal and cellular responses to irradiation.

High-LET Radiations as the Principal Focus

High-LET radiations should be the principal research focus, with low-LET radiation effects used for control studies, for the advantage of biophysical systematics which derive from LET dependencies, and for relating results to the available literature. For mechanistic studies, high-LET radiations, for example, charged particles having narrow ranges of LET, should be useful. Energy localization and transfer from one cellular component to another must have a high priority. More chemical change occurs per energy deposition event with a high-LET radiation than with a low-LET radiation, and the multiple ionizations of high-LET radiations occur almost simultaneously. High- and low-LET radiations cause different yields of lesions in cells (or molecules), and studies with high-LET radiations offer the possibility of a new level of observation and understanding of radiobiological effects. One interesting aspect is that high-LET radiations are the only way to obtain large numbers of coordinated ionizations in a polymer (DNA, protein) or cell. Multiple ionizations probably can result in secondary reactions which are different from those following single ionizations. The study of multiple ionization events may open a new field of chemistry as well as biology.

Radiation Chemistry

In spite of the successful research with elementary experimental systems, as yet it is not possible to identify a specific series of reactions, or a specific pathway, leading from energy deposition to any unique biological endpoint. Additional uncertainty is presented by cells, in which conditions are likely to modify reactions observed under simplified noncellular conditions. We need to determine how the kinetics and reaction paths are modified by membranes, by other organized structures, and by the relatively high total concentrations of solutes in cells. How do excitations and ionizations in a cell cause DNA strand breaks, mutations, or any other cellular endpoint? What biophysical and molecular reactions occur in the cell during this stage? These are examples of important questions yet to be answered.

In addition, we have very little information on the radiation chemistry of high-LET radiations. From results on water radiolysis obtained 30 years ago with alpha particles we know that yields of $\text{OH}\cdot$ and e_{aq}^- (radical products) are much lower than with low-LET radiation, and yields of H_2O_2 and H_2 (molecular products) are larger. Little is known even for noncellular conditions about differences in radical and molecular product yields on radiolysis of molecules other than water. Virtually nothing is known about these reactions in cells. Basic studies are needed to fill this gap.

As stated earlier, useful biophysical/molecular studies must take into account the heterogeneity of cells. Each cell may be considered as a highly compartmentalized suspension containing a fairly concentrated solution or sol phase, numerous interfaces, membranes, and fibrous structures, probably no free water, numerous subcellular structures, and a bounding membrane. Thus, the identity of reaction products, durations and directions of reactions, and whether biological damage is the result of direct or indirect action may be determined by surface, interfacial, and other structural cellular features. Better experimental systems for studies of molecular reactions as they might occur in cells include micelles in aqueous or organic media, vesicles, surface films, and various complexes of DNA with proteins and other materials. Because of the relatively high concentration of solutes in the cell, topics such as degree of hydration of macromolecules and counter ions associated with macromolecules should be investigated along with their role in determining conformation, function, and response to damage.

It probably takes about 10^{-14} seconds after being hit by radiation or attacked by a free radical for a molecule to begin to change its conformation from a normal form to forms that are incompatible with its usual biological function. Therefore, the very early events following a specific energy deposition need to be elucidated.

Photochemical phenomena are important in studies of the molecular and biophysical aspects of radiobiology. A characteristic of radiation-induced phenomena in noncellular and cellular systems is that numerous reactions occur simultaneously, and it is often difficult to isolate a particular reaction for observation. Photochemical studies provide a useful way of accomplishing at least a partial separation of some phenomena. Photochemical investigations permit the study of ionization or excitation to known energy levels, the decay of excited states, radical recombination, energy transfer processes, changes in molecular conformation, and, with

intense sources such as synchrotrons, excitation to upper excited levels and coordinated multiple excitation of polymeric molecules, such as DNA and proteins. These states and processes occur with ionizing radiation, but they are difficult to observe because many competing processes occur on a short time scale.

Development of Methodology

Chemical Techniques. New technologies may be of use in radiobiology. One example is the scanning tunneling microscope, an instrument which can resolve individual atoms. It has been developed and used extensively in the physical sciences, and successful attempts to examine DNA have been reported. The development of a technique to examine DNA, proteins, and other biological materials before and after various treatments should be attempted with the hope of making fundamental observations at the molecular and atomic level of alterations, such as base changes in DNA, conformational changes, and cross-linkage between DNA and proteins.

A second opportunity is presented by the synchrotron. This device can provide ultra-high fluxes of photons which facilitate the study of excited states, their transitions, and their reactions.

Accelerators of heavy ions, including protons, deuterons, and helium ions, should be used to investigate the effects of variation of LET on early radiation events in model systems and cells. Such investigations will make possible the analysis of ionization density as a factor in radiation responses.

Other tools that should be investigated include laser spectroscopy and fluorescence imaging which permit direct observation of reactions in single cells. For certain purposes, ultra-sensitive detection and measuring techniques must be developed, utilizing the full resolving power available, within physical constraints, to obtain the maximum possible molecular detail.

Cellular and Biochemical Techniques. Further research is needed to identify the lesion induced in DNA which is biologically most relevant. In particular, emphasis should be placed on determining whether high-LET and low-LET radiations produce lesions which differ from each other in quality, quantity, spatial distribution, or the ability of cells to repair them correctly. It would be of major value to identify lesions which are uniquely or preferentially produced by neutron irradiation and which may be causal factors in the induction of cellular endpoints.

For the elucidation of the mechanisms by which DNA lesions lead to cellular responses, there is a need for the development of more sensitive assays for the detection of the different types of damage. Single strand breaks can be detected with greater sensitivity using the alkaline unwinding-hydroxyapatite technique (Rydberg 1980) or alkaline filter elution (Kohn et al. 1981) than using alkaline sucrose sedimentation. However, both of the more sensitive techniques detect classes of lesions, including actual radiation-induced strand breaks as well as lesions which are converted to strand breaks under the alkaline conditions of the assay. For some studies of the physiological consequences of DNA damage resulting from low doses of low- or

high-LET radiation on cell populations, it will be advantageous to determine the yield of damage in single cells. Techniques, such as those employing flow cytometry or single-cell microelectrophoresis, should be further developed for such studies (Ostling and Johanson 1984; Olive 1989).

Because of the evidence suggesting the importance of double strand breaks as causative lesions in cellular responses, emphasis should be placed on newer methods for determining the yield complexity and repairability of double strand breaks at relevant doses, primarily utilizing pulsed-field or alternating-field gel electrophoresis (Schwartz and Cantor 1984). The advantage of these techniques is that they allow the measurement of very large DNA molecules, permitting strand breakage to be recorded at lower doses than those needed for comparable measurements with traditional techniques. At present, the individual intact chromosomes of the yeasts *Saccharomyces cerevisiae* (largest chromosome, 3 Mb [million base pairs]) and *Schizosaccharomyces pombe* (largest chromosome, 6 Mb) are easily resolved. Although intact mammalian chromosomes are too large for direct measurement of DNA size by present techniques (largest chromosome, ca. 100 Mb), certain modifications permit measurement of mammalian cell chromosomes. For example, large DNA fragments generated by treating cells contained in an agarose plug with infrequently cutting restriction endonucleases can be sized. Alternatively, DNA double strand breakage can be quantified indirectly in terms of the dose-dependence for the entrance of DNA fragments from the agarose plug into the gel (Blöcher et al. 1989). With further development, for example, clearly distinguishing between the effects of breakage and the effects on release of DNA from complexes with other cellular components, pulsed or alternating field electrophoresis should offer comparative information on the effects of neutrons and low-LET radiations with respect to the formation and repair of double strand breaks.

Sensitive measurements of chromosome breakage are being developed using fluorescent cell sorters. In this case, the loss of a unique chromosome peak is taken as an indication of a break. These methods require further development, but they may be useful for the detection of very low levels of damage in mitotic chromosomes. In addition, the technique of premature chromosome condensation permits the detection of damage to chromosome structure and its repair; these measurements would be particularly relevant to high-LET damage.

Improvements are also needed in the methodology for detecting DNA base and sugar lesions and covalent linkages between bases or between amino acids and nucleosides using, for example, techniques such as high performance liquid chromatography and gas chromatography, combined with mass spectrometric detection systems (Dizdaroglu 1986). Additionally, immunochemical approaches, using antibodies against individual lesions or classes of lesions, are becoming more sensitive (Fuciarelli et al. 1985). Once these approaches are adequately characterized, comparative analyses of low- and high-LET radiations should yield valuable information.

During the past 20 years, significant progress has been made in the fields of genetics, immunology, and molecular biology. It is essential in planning molecular-level experiments in radiobiology to include concepts and knowledge of cells and cell function from these fields (see, e.g., Thacker 1986; Elkind et al. 1990).

Identification of Clustered Lesions

Because the neutron dose is deposited predominantly by densely ionizing tracks, for a given dose there is a lower probability of an ionization occurring in or near a given molecule than there is with low-LET radiations. But the neutrons have a greater probability of producing dense clusters of multiple ionizations and may even produce local events which are virtually impossible with low-LET radiations. The greater biological effect of neutrons may well be due to these dense clusters. Studies of the radiation chemistry of neutrons should therefore include products which are formed by such clusters.

Clustered lesions might be expected to be repaired with greater difficulty than single independent lesions. Assays for complex damage of this kind do not exist, other than those for the class of lesions measured as double strand breaks. Full descriptions of DNA damage resulting from all types of ionizing radiation, including neutrons, will require analyses of subclasses of double strand breaks and other lesion clusters and the manner in which they are processed by cellular metabolic systems.

Effects of Conformation of Cellular Targets

Changes in the conformation of macromolecules in response to ionization, excitation, and free radical formation very likely cause significant changes in the ability of macromolecules to carry out their usual function. Examples where conformation is vitally important occur in immune reactions, DNA replication and repair, DNA transcription, and other metabolic reactions, as well as in maintenance of essential cellular structures.

There is a need for greater understanding of the role of the structural organization of the DNA target in determining both the formation and the repair of radiation-induced DNA lesions. This area needs to be investigated using both cellular and cell-free systems.

In spite of the inadequacy of most cell-free constructs for studies of the effects of radiation on intracellular chromatin, some constructs may be useful for defining the relative sensitivity of particular sequence classes or structures, for example, condensed chromatin in comparison to histone-depleted extended structures, as described by Heussen et al. (1987). In addition, modern biotechnology presents some new opportunities for the acquisition of useful information. Specifically designed plasmids carrying sequences with specialized properties may facilitate the determination of the role of non-B-type DNA structures or the role of sequence-specific protein binding in modifying those structures and their sensitivity to irradiation. Assays for the distribution of lesions within such plasmids will have to be developed, including the use of hybridization probes for specific sequences which may suffer greater or lesser damage than the bulk of the DNA.

The precise location of some lesions may be determined using sequencing methodologies with DNA fragments of defined sequences. These techniques have been used successfully to define the structure of strand breaks and other lesions produced by gamma radiation and to observe the ability of some repair enzymes (e.g., Doetsch et al. 1987) to recognize lesions

within the defined sequence. In addition, they have been used to determine which lesions can serve as templates for replication by DNA polymerase, which ones block further DNA replication, and which ones are potentially mutagenic (Ide et al. 1985). The power of the sequencing methodologies can help to define precisely the chemistry, base specificity, if any, and the distribution of DNA lesions produced by neutron irradiation. In addition, specific types of damaged nucleotides may be introduced by chemical techniques or by genetic engineering methodologies in order to mimic radiation-induced damage and to study its repair or consequences as a function of its location within a defined sequence or relative to another lesion.

The study of cell-free plasmids or chromatin fractions in an environment which mimics the intracellular milieu will require the use of high total doses of radiation, but it may reveal whether high- and low-LET radiations produce lesions which differ in type, amount, oxygen dependence, or distribution in these systems.

The investigation of the influence of DNA/chromatin structure and nuclear architecture on DNA damage and its processing should proceed with the development of assays for monitoring structural changes and damage levels and processing within unique DNA domains in cells. Implicit in this program is the need to develop procedures for the identification of clusters of lesions and large-scale lesions, such as disaggregation of chromosomal loops or modified interactions of chromosomes with the nuclear matrix and lamina, as exemplified by the fluorescent halo assay (Roti Roti and Wright 1987).

Relationship of Molecular Studies to Radiobiological Parameters

Damage produced in both cellular and cell-free systems should be examined from the point of view of fundamental radiobiological parameters, such as effects of dose rate, dependence on oxygen, dependence on radiation energy, and dependence on LET. Such studies can help direct attention to the molecular lesions most relevant to the important biological endpoints. For example, cellular effects of high-LET radiations display a lesser dependence on oxygen than do effects of low-LET radiations. Furthermore, chemical damage, including single strand breaks in DNA, which result from single ionizations, do not show the dependence on LET observed for cellular effects and can be eliminated on this basis as relevant to the cellular effects (Frankenberg-Schwager 1990).

Enzymatic Processing of Initial Damage

The processing of different types of radiation-induced damage needs to be investigated. Both cellular and cell-free systems should be employed for these studies, as well as for those above.

Some of the cell-free DNA systems described above should be useful for examining the recognition of various lesions by specific endonucleases. It would be important to determine, for example, whether or not endonucleases which cleave DNA near base damage produced by

low-LET radiation are as efficient when confronted with damage produced by neutron irradiation. Closely opposed sites within clusters of lesions may not be processed as readily as single sites.

Processing of lesions may differ greatly within cells exposed to radiations of different qualities. For example, a higher fraction of DNA double strand breaks remains refractory to repair following high-LET as compared to low-LET radiation (e.g., Peak et al. 1991) and are preferred candidates for critical lesions responsible for differences in cellular responses to high- and low-LET radiations. Other types of lesion clusters or large structural changes in nuclei might have similar repair characteristics, and current assays do not distinguish between restoration of normal structure and simple removal of the damage. Assays to further the molecular characterization of repair need to be developed. This includes isolation of additional enzymes that catalyze repair processes and elucidation of mechanisms that regulate these processes.

Cells that are radioresistant with respect to the lethal effects of radiation may be useful for elucidating the types of damage which the cell does not normally repair. Clues as to the cellular processes required for faithful repair of such damage may be obtained by comparison of related repair-deficient and repair-competent cells and by selective blockage of segments of cellular metabolism, such as DNA replication, poly (ADP-ribose) synthesis, thiol metabolism, and the pentose-phosphate shunt, that is, pathways implicated in enzymatic repair processes.

The role of modern molecular biology in these investigations will be crucial. The identification and cloning of new enzymes for repair of DNA lesions, the elucidation of the detailed mechanisms for the repair of DNA, the alteration of cellular properties by the introduction of specific cloned genes and gene products, the selective deletion of candidate gene products using anti-sense technologies, and the examination of cellular and tissue specificities for DNA repair mechanisms in transgenic animals are examples of the opportunities provided by developments in molecular biology.

Alterations in Gene Regulation by Radiation

In addition to the stimulation of mechanisms for the direct repair of DNA damage, ionizing radiation induces a metabolic response with features similar to stress responses induced by heat and other forms of oxidative stress (Weichselbaum et al. 1991). Exposure of cells to radiation activates signal transduction mechanisms involving membrane phospholipid breakdown and protein kinase C. The early signalling events can result either from DNA damage or from free radical reactions in cellular membranes and are followed by a program of genetic events that alter the cellular levels of specific RNAs and proteins. Among the genes whose expression is enhanced in irradiated cells are the early response genes *c-fos* and *c-jun*, genes for protein kinase C, beta-actin, and tissue plasminogen activator, as well as numerous unidentified genes (e.g., Woloschak and Chiang-Liu 1990; Woloschak et al. 1990a, b; Boothman et al. 1991; Papathanasiou et al. 1991). Significant increases in the levels of certain mRNAs have been observed in cells exposed to as little as 1 cGy of fission spectrum neutrons or 6 cGy of gamma

rays (Woloschak and Chiang-Liu 1990; Woloschak et al. 1990a, b). The activation of genes, such as those for protein kinase C and tissue plasminogen activator, which have been implicated in cellular transformation processes, suggests that the modulation of specific gene expression is an important component of cellular transformation by ionizing radiation. In order to understand how radiation produces early cellular changes leading to transformation, further elucidation of this response is needed, with emphasis on identification of the genes involved and their roles in cellular responses to radiation, the mechanisms of regulation of the identified genes, and delineation of the dependence of elements of the response on LET.

 

Chapter VI

CELLULAR AND GENETIC STUDIES*

INTRODUCTION

For studies with fast neutrons, such as fission spectrum neutrons, at a cellular level, the endpoints of general interest are killing, mutation, neoplastic transformation, and altered differentiation. Because the expression of phenotypic changes requires viability, an understanding of killing is included among the preceding even though the mechanism of lethality may involve essential differences from the other endpoints.

The long-term effects of exposure to low doses of ionizing radiation result, at least in part, from genetic damage to the cells which are exposed. The genetic damage results primarily in mutations and/or in oncogenic transformations. Although risks such as these have been enumerated in animal and cellular systems, further advances in the understanding of the biology of these changes require new insight into the molecular mechanisms connected with the multiple steps which occur from the initial DNA damage to the expression of an altered cellular phenotype.

Neutron radiobiology cannot be divorced from radiobiology in general. Differences in degree and probably in kind are likely between the effects of low-LET radiations like x and gamma rays and those of fission spectrum neutrons. Nonetheless, in broad terms it is reasonable to expect the same micro- and macrodosimetric principles to apply, the same molecules to constitute the principal targets, the same intracellular structures to be affected, and the same or at least similar pathways to be involved when a given endpoint is expressed.

Neutron radiobiology is also not separate from high-LET radiobiology. However, because of the spectrum of secondary events plus the concomitant exposure to gamma rays which results from energy deposition by neutrons, it is also not correct to assume that a beam of high-LET charged particles is biologically equivalent to a beam of neutrons. Still, in unravelling the biological contributions that may come from different parts of a complex beam, track-segment studies with monoenergetic high-LET particles can be useful.

Most radiobiological effects produced *in vivo* have cellular origins. The discrete and random absorption of ionizing radiation, as well as the magnitude of energy deposition events, means that initially all molecules and anatomical structures are vulnerable to radiation damage. Still, because there is a hierarchy of targets which when damaged culminate in observable cellular changes, it is important to identify those lesions, targets, and subsequent processes of greatest relevance to the endpoint in question.

The principal end effects of cellular radiobiology—killing, mutation, transformation, and altered development, including altered differentiation—also are changes of concern in other branches of cell biology. Although other differences are involved, other physical, chemical, and

* The major part of the material in this report was prepared prior to June 1991.

biological agents can produce the same or similar cellular changes. In addition, because radiation-induced endpoints frequently are heritable and probably involve changes in the genome and/or its expression, radiobiology is concerned with cellular elements which have large biological amplification.

HIGH- VERSUS LOW-DOSE RADIOBIOLOGY

From the point of view of radiation protection, it is necessary to know the degree of risk per unit dose due to low doses, which are frequently protracted over long periods of time compared to characteristic biochemical reaction times, cell-cycle times, or even the life spans of essential cells in the body. Although the emphasis in radiation protection is on low-dose effects at molecular, cellular, and organismic levels, in order to understand the mechanisms of radiobiology, it may be necessary also to understand the effects of higher doses.

An example of the foregoing is the variation in killing sensitivity as cells progress through their cycle. Because killing, or survival, is a high-frequency event, it is readily measured and sufficient data for analysis are available. Other radiation-induced changes also have been shown to depend upon cell-cycle position; and other agents also induce cycle-dependent effects. In the dose region from zero to 50-100 cGy, with the exception of mitotic cells perhaps, cell-cycle variations in survival are not readily detected. This apparent nonvarying response could be interpreted to indicate that the mechanism of lethality does not depend on where, in its growth cycle, a cell is when it is irradiated. Accordingly, it could be inferred that the expression of the molecular lesions responsible for inactivation are also cell-cycle invariant and, further, that only those lesions evident in the dose range from zero to 50-100 cGy are relevant to killing.

However, it is known that mid-to-large doses (depending on the cells, from 100-1000 cGy) result in significant variations in survival through the cell cycle. This variation suggests that the mechanism of killing, and/or the lesions involved, vary as a cell progresses from mitosis to mitosis. Further, the molecular changes due to mid-to-large doses are more readily measured, and, in a given instance, might not be detectable after low doses.

The preceding indicates that the radiobiology of killing, for example, requires an understanding of more than what may appear to ensue only after low doses. It follows that similar considerations apply to radiation-induced, low-frequency events like mutation and transformation. Furthermore, because of problems inherent in the detection and measurement of events at low frequencies, it may be a practical necessity to seek data from doses which lie outside of the range of programmatic interest.

RELATIONSHIP BETWEEN ENDPOINTS

Ionizing radiation is effective in breaking chromosomes and producing chromosome aberrations. This property plus the heritable changes produced by radiation are the bases for seeking similarities in mechanisms for different endpoints. Accordingly, survival, which is easy

to measure for dividing cells, is frequently used as a means of detecting so-called "genotoxic agents" which presumably would be potential mutagens and/or carcinogens. Similarly, a neoplastic transformation frequently has been assumed to be a manifestation of a somatic mutation. Thus, killing, mutation, and transformation endpoints mechanistically are considered to have elements in common, which also suggests that in repair-proficient cells, at least, repair/misrepair processes are similar for the three endpoints. The systematics of radiobiology (e.g., dependencies on dose, dose protraction, LET, etc.) should be of value in establishing the possible connections between these endpoints.

An additional issue relative to endpoints is the difference in tumor induction rates between species, in a given species between strains, and in a given strain between tissues. The unifying feature in each case is a manifestation of a loss of the capability to regulate growth. But, it is not clear that the process of initiating the cancer, as well as its frank expression, are the same in all instances. Although the properties of radiation-induced tumors in a given tissue are not qualitatively different from those tumors which may have different etiologies, it is not known if, or to what degree, the steps in the overall process are the same (Elkind et al. 1990).

At a cellular level, the following questions can be addressed.

- (1) For a given cell system, are the mechanisms of induction the same with different inducing agents, for example, physical versus chemical and other agents?
- (2) For a given inducer, like neutron irradiation, to what extent are the mechanisms the same among different cell systems?
- (3) Do different inducers produce interactive effects, that is, protective, additive, or synergistic effects?

GENETIC MECHANISMS

Neoplastic Transformation

The tumorigenic and molecular properties of retroviruses have served to identify a class of genes, the proto-oncogenes, in mammalian cells which appear to play a role in oncogenesis and, therefore, in the neoplastic transformation of cells. The gene products, their function, and, consequently, the normal roles of these genes are not entirely understood, but it is believed that radiation and oncogens in general affect proto-oncogenes or their expression via one or more of the following mechanisms.

- (1) Point mutation — One or a few base pairs are changed resulting in an altered gene product, which results in the loss of the regulation of growth (e.g., Tabin et al. 1982; reviewed by Barbacid 1987).

(2) Gene amplification — The copy number of a proto-oncogene is increased by as much as an order of magnitude or more from the normal number. As a result, the overproduction of an otherwise normal gene product leads to a loss of growth regulation (reviewed by Bishop 1983a, b).

(3) Altered regulation — A change occurs in the control element(s), acting in *cis* or in *trans*, of a proto-oncogene, thus significantly altering its rate of transcription. This change could lead to the over- or underproduction of a gene product which, hence, could affect the control of growth.

(4) Viral induction — Control elements of viral DNA may activate neoplastic potential by becoming transposed into a position from which they can alter the expression of a normal proto-oncogene. Additionally, when retroviral DNA is inserted into the genome of a mammalian cell, proto-oncogenes can be mutated in the process. A third potential role for retroviruses in tumorigenesis is via transduction. A mutated proto-oncogene may become incorporated into a viral genome upon the integration of the latter into a cell's genome. Alternatively, the proto-oncogene may be mutated after transduction. In either case, when the virus becomes expressed, it may transform additional cells by inserting the mutated oncogene into the recipient's genome.

(5) Genetic rearrangement — A normal proto-oncogene may be transposed into a normal but transcriptionally active region of the same chromosome, or some other chromosome, such as the region in the genome of a B-type lymphocyte which is involved in antibody production. This shift could result in the overproduction of the gene product of the proto-oncogene with the consequent loss of growth control. The frequently observed "break points" and chromosomal rearrangements in the karyotypes of human tumors could be a reflection of chromosomal rearrangements as precursors to proto-oncogene expression or overexpression (e.g., see Yunis 1983; Rowley 1984).

(6) Inactivation or loss of suppressor genes — In some instances, it has been shown that changes connected with a pair of allelic, autosomal genes can result in uncontrolled growth when the normal products of both genes are lost. The product of a single gene of such a pair is sufficient to suppress unregulated growth. At a molecular/chromosomal level, one altered gene may be inherited, as in familial retinoblastoma (see Klein and Klein 1985; Benedict et al. 1987; Klein 1987), with the consequence that a single change, or loss of function, in the allelic gene can result in the neoplasm. In an increasing number of tumors, neoplastic growth has been associated with the second change effected by the loss of a (presumptive) suppressor gene located in a chromosomal region that has become deleted and, therefore, is no longer heterozygous with a corresponding region in normal cells (see Weinberg 1991).

The foregoing illustrate how varied the molecular mechanisms may be which may culminate in an aberrant regulation of growth.

In addition, there is evidence that tumor induction is a multistep process and that the involvement of two or more oncogenes may be required to effect the multiple steps (e.g., see Weinstein et al. 1984; Bishop 1987). Consequently, in principle, more than one genetic change may be required.

Oncogenes and Radiation-Induced Changes in Cells and Tumors

Oncogenes in chemically induced animal tumors have been studied extensively. There has been considerably less effort to study or identify activated oncogenes after radiation-induced cell transformation, and few of these efforts have dealt with oncogene activation by high-LET radiations. Some examples of the association of activated oncogenes with radiation-induced transformation are the following.

The first observation of an association of a mutationally activated *K-ras* oncogene with gamma-radiation-induced tumors was reported by Guerrero, Pellicer, and coworkers (Guerrero et al. 1984a, b). A compilation of mutated oncogenes found by these investigators revealed that only *K-* and *N-ras* were activated in gamma-radiation-induced tumors (Diamond et al. 1987; Guerrero and Pellicer 1987). The mutation involved was frequently a G to A transition in the second base of the 12th codon. These investigators also reported the induction of approximately 20 thymic lymphomas in RF/J mice by neutron irradiation. As studied by oligonucleotide mis-match hybridization, none of the neutron-induced tumors contained the G to A transition as in gamma radiation-induced tumors, suggesting that differences at an oncogene level can result with different radiations. However, not all of the thymomas induced by x radiation contained a mutated *ras* gene.

Activation of both *c-myc* and *K-ras* oncogenes has been observed in primary skin tumors induced in male Sprague-Dawley rats exposed to single or to multiple doses of 8 to 16 Gy of 0.8 MeV electrons starting at 4 weeks of age (Sawey et al. 1987). Activated oncogenes have also been observed in lung tumors of beagle dogs given single aerosol exposures of $^{238}\text{PuO}_2$, $^{239}\text{PuO}_2$, or $^{239}\text{Pu}(\text{NO}_3)_4$ (Frazier et al. 1987). DNA from these tumors was active in the NIH 3T3 focus-formation assay. The dominant-acting oncogene(s) in these transformants was variously identified by restriction fragment length polymorphism as *src*, *myc*, *H-ras*, *K-ras*, or *erb-B*. Although the most prominent altered oncogene in plutonium-induced tumors was *K-ras*, an activated *N-ras* was characteristic of gamma-radiation-induced myeloproliferative disorders in the same animals.

Altered *K-ras* and *c-myc* genes have also been observed in association with the *in vitro* transformation of WI-38 human lung fibroblasts by gamma radiation (Mizuki et al. 1985). In the transformed cells, the *K-ras* gene was amplified 3-fold with a concomitant increase in expression. Although no gross rearrangements of *c-myc* were observed, there was increased expression of this gene in the transformed cells.

There is also evidence that other as yet undefined oncogenes may be involved in the oncogenic process induced by the irradiation of Syrian hamster embryo cells and mouse $\text{C}_3\text{H}10\text{T}\frac{1}{2}$ cells *in vitro* (Borek et al. 1987). In an increasing number of both rodent and human

tumors, appreciable progress has been made in identifying the oncogenic changes and chromosomal rearrangements associated with neoplastic growth (see Elkind et al. 1990).

Somatic Mutations

The similarities between the relative efficiencies of many environmental toxins and the mutagenesis and/or oncogenesis that they may give rise to suggest that the mechanisms of mutagenesis could be studied to advantage even in genes that are not known to be directly connected with oncogenesis. Ionizing radiation is capable of producing all of the known types of mutations, that is, point mutations (transitions, transversions, and frame shifts), small and large deletions, gene amplifications, gene translocations, and other gross rearrangements. However, in comparison to alkylating agents and ultraviolet radiation, ionizing radiation is a poor point mutagen, producing alterations in gene loci predominantly via large-scale rearrangements (Cox and Masson 1978; Thacker and Cox 1983). The percentage of recovered mutants which carry simple point mutations is generally low and varies with the ability of the cell system to detect more extensive types of lesions (reviewed by Thacker 1985).

The mutational frequencies observed may be influenced by several factors such as the following.

- (1) A specific gene, or part of a gene, is a small target relative to that for lethality and is one reason contributing to the low yield of mutations due to ionizing radiation.

- (2) The position of the gene in question, relative to chromosomal "hot spots" for rearrangements or deletions, may influence mutational frequencies.

- (3) The location of the gene—that is, on a homozygous or hemizygous chromosome, a heterozygous region of an autosome—has an influence on mutational frequencies. The measurement of the mutagenicity of various chemical and physical agents in cultured mammalian cells has generally relied upon the determination of the frequency of forward mutations which inactivate genes coding for nonessential products. Target genes of choice, in which the inactivation of one allele results in a measurable phenotypic change, are usually found in the heterozygous state, in hemizygous regions of a chromosome, or are codominant. Clastogenic agents, such as ionizing radiation, are generally more mutagenic at heterozygous loci than at loci located in the hemizygous regions of a chromosome (e.g., Evans et al. 1986). When the hemizygous condition applies, the latter observation is due to the poor recovery of mutants which harbor multilocus lesions in essential genes because such mutants would not be expected to survive. As a result, the mutation frequencies measured are lower than otherwise expected, and the spectrum of mutants recovered can be expected to be skewed toward point mutations, small deletions, and rearrangements. Also, the study of diploid homozygotic targets is confounded by the difficulty in detecting the loss of one allele in the presence of a second, functional, wild-type allele (Thacker 1985; Evans et al. 1986).

Thus, in seeking to understand a genetic basis for radiation-induced transformation and how it may relate to somatic mutation, it is necessary to understand that the molecular features may differ in detail (see Elkind et al. 1990).

Although a principal objective should be to understand, at a molecular level, how radiation induces cancer and how and why the induction depends upon the radiobiological parameters of dose, dose protraction, and the quality of the radiation, it has to be recognized that the molecular biology of oncogenesis is not understood well enough to permit a straightforward examination of radiobiological variables and parameters. Because of some of the unique properties of ionizing radiation, as well as programmatic needs, in order to be effective a program of research should seek to understand how genotoxic agents in general produce the cellular effects which culminate in mutagenesis and oncogenesis. Chemical oncogenesis can be a foil for radiation oncogenesis and *vice versa*.

ENHANCED NEOPLASTIC TRANSFORMATION BY PROTRACTED EXPOSURES OF FISSION-SPECTRUM NEUTRONS

Although the magnitude of an effect produced by a low-LET radiation is generally reduced if the exposure to a given dose is protracted (for data on neoplastic transformation, e.g., see Han et al. 1980; Hill et al. 1984b), the reverse effect has been reported for reactor-generated neutrons. When C₃H mouse embryo cells, designated 10T^{1/2}, were exposed to reactor neutrons at reduced dose rates (Hill et al. 1982, 1984a)—or to multiple, daily high-dose-rate exposures (Hill et al. 1985)—an enhanced frequency of transformation was observed. In the latter studies, the transformation endpoint was focus formation on a background of growth-arrested nontransformed cells (Reznikoff et al. 1973). This effect was only observed after small-to-moderate neutron doses, and at doses greater than about 20 cGy the enhancement due to protraction was progressively lost. Borek and Hall (1984) reported that for x irradiation for doses between 0.1 and 0.5 Gy, fractionation enhanced the transformation incidence; while for relatively high doses in excess of 0.5 Gy, fractionation led to a sparing effect for transformation.

Since these initial reports, an enhanced neutron-induced rate of transformation was confirmed in a number of instances using different cells, endpoints, and sources of neutrons (see Elkind 1991a, b). However, confirmation was not obtained in other studies performed with 10T^{1/2} cells and fission-spectrum neutrons (Balcer-Kubiczek et al. 1988) or high-LET charged particles (Hieber et al. 1987; Bettega et al. 1989). It should be noted that there are differences of opinion regarding the methodology and significance of transformation assays, considering that even plating procedures and serum conditions can affect the expression of malignant transformation.

A model developed by Elkind (1991a, b) attempts to explain the preceding apparently contradictory results. Because of technical considerations connected with the changes in the properties of cells as they approach and then leave mitosis and the limited range of the maximally-effective charged particles used by Hieber et al. (1987), this model is based upon a "window" of responsiveness of cells in or near mitosis. It is assumed that the cells in this window have these properties: (1) an enhanced sensitivity to neoplastic transformation; (2) an

enhanced sensitivity to killing; and (3) a dose-dependent block to the progression of cells into the window, that is, in late G₂ phase. The model of Rossi and Kellerer (1986) developed to explain the data of Hill et al. (1980, 1982), and its further application by Brenner and Hall (1990), is essentially different because it does not specifically invoke the special properties of mitotic cells in reference to killing, and radiation-induced division delay in G₂ (Elkind 1991a, b). Further, in the latter model, cell kinetics without dose-protraction-related repair is a general radiobiological feature of the response of cells exposed to a high-LET radiation, whereas kinetics plus repair dominate when exposures to low-LET radiations are protracted (e.g., Han et al. 1980; Hill et al. 1984b).

RESEARCH NEEDS

Technical Developments

In order for changes produced in cells to form a basis for studies of oncogenesis, it is important to improve the systems available for quantitative work.

Cell Types and Criteria. At present, only a few cell systems have been used effectively for quantitative studies of radiation-induced neoplastic transformation. Principal among these are primary, and in some instances secondary and tertiary, cultures of Syrian hamster embryo (SHE) cells (Borek and Sachs 1966); C₃H mouse embryo cells designated 10T $\frac{1}{2}$ (Reznikoff et al. 1973); NIH BALB/3T3 cells (Little 1979); and human HeLa/normal fibroblast hybrid cells (Redpath et al. 1990). In each instance, the cells which are assayed are fibroblasts. With SHE cells, the transformed phenotype is identified by the altered appearance of a colony of cells growing well separated from other colonies; whereas with 10T $\frac{1}{2}$ and 3T3 cells, the formation of a focus of transformed cells is identified by its altered morphology after it forms in the midst, or on top, of a confluent layer of normal-appearing cells. With the human hybrid cells, HeLa/normal fibroblast, neoplastic transformation is made evident by the appearance of a new protein, intestinal alkaline phosphatase, on the plasma membrane.

The mechanism underlying each of the preceding criteria has not been worked out, but it could involve a decoupling, or at least a modulation, of cell-to-cell communication in the case of 10T $\frac{1}{2}$ and 3T3 cells (Mehta et al. 1986), which is normally mediated via gap junctions (Lowenstein 1987). Also, there are qualitative as well as quantitative differences between the results obtained with these cells. Cell systems involving the use of epithelial cells are needed because most tumors are carcinomas, but, in addition, more types of transformation assays are also needed with fibroblasts and epithelial cells. Further, as a parameter influencing the yield of mutation and transformation endpoints, the relevance of the proliferative status of a population should be examined. A number of radioresponsive tissues of the body consist largely of noncycling cells. Growth-arrested cells *in vitro*, due to cell-to-cell communication or to cell crowding/medium insufficiency, may not be equivalent to noncycling cells *in vivo*. Further, *in vitro* systems should be developed to simulate the role of growth stimulation and the importance of normal cell turnover versus compensatory radiation-induced repopulation in tumorigenesis.

Assays of Tumorigenicity. In addition to rodent cells, it is important to develop more systems which come from other animals, including people. Here, too, the objective would be to establish generalizations, but also to seek parallels between the results obtained *in vitro* and those obtained *in vivo*. Although most of the radiation studies with animals have been performed with rodents, some data are available from studies with canines. To establish that particular cellular changes in, for example, human cells are indicative of the phenotype of transformation, *in vivo* assays in immune-deficient mice or an equivalent procedure should be employed. This approach also would offer the opportunity to inquire if different selection pressures alter the properties of cells which yield tumors from those which produce a particular endpoint *in vitro*.

Media and Sera. Quantitative studies with mammalian cells in culture usually require that a complex medium plus serum must be used. Of the various sera which are commercially available, fetal bovine serum is frequently preferred or even required. At one extreme, a given lot of this serum might be frankly toxic to the cells under study and, at the other, all of the essential properties of the cells may be readily demonstrable, including reproducible yields of the transformation endpoint with various inducing agents. Between these extremes, various gradations of the properties of the serum are encountered (Elkind 1991b). Observations such as these suggest that subtle differences exist between the lots of serum of the same type. The observations of Hsiao et al. (1987) indicate that fetal calf serum can contain a factor(s) which considerably enhances its effectiveness in expressing the transformed phenotype compared to calf serum. Similar studies could be undertaken to identify the essential chemical principal(s) in serum that promote, or suppress, the transformation endpoint.

Therefore, it is important that the essential nutritional and other factors required for a given assay system should be sufficiently well understood so that they can be adequately controlled. Serum, with its host of ill-defined ingredients, optimally should be replaced by a specific chemical mixture to permit the use of a completely defined and therefore controllable growth medium.

Oncogenes

DNA transfection has been used to suggest that, in a number of instances, chemically-induced tumors result from mutated proto-oncogenes.

Molecular Characterization of Genetic Controls of Oncogenesis. In the past decade, significant advances have been made in the identification and characterization of tumor-associated oncogenes and their protein products. Cellular oncogenes have been identified, initially by their partial homology to the transforming genes of retroviruses, and subsequently by their ability, once activated, to be serially transferred from transformed cells to certain normal cells (usually NIH 3T3 mouse fibroblasts), resulting in the transfer of the oncogenic properties to the recipient cell. Activation of cellular oncogenes may occur spontaneously at very low frequency or may be stimulated by exposure to oncogenic chemicals, to radiation, or to viruses. Although the mechanisms by which one or more of the oncogenes participate in the

oncogenic process remain essentially unknown, there is considerable evidence that many of the oncogenes play a role in untransformed cells in the control of normal cell growth (Bishop 1983a, b; Land et al. 1983).

Information available on the relationship of the activation of oncogenes to radiation-induced neoplastic transformation is limited; general patterns have not as yet emerged as the following illustrate.

(1) Some data are available on the stage in the development of radiation-induced tumors at which a particular oncogene becomes altered, activated, and/or expressed. There is some agreement that *ras* genes participate at a late stage of tumorigenesis, but stage dependence has not been studied in sufficient detail during the progression of radiation-induced tumors. A number of model systems, which may be amenable to this type of analysis, are being developed. For example, the development of mammary tumors in mice has been studied by Ullrich; preneoplastic lesions were detected and cultured *in vitro* from tissue treated *in vivo* (Ethier and Ullrich 1982; Ullrich 1986). Also, Terzaghi-Howe and collaborators have been developing an *in vivo/in vitro* system for studying neoplastic changes in rat tracheal epithelial cells (e.g., Terzaghi-Howe 1989), and, similarly, Reznikoff and collaborators have been working on a human urinary tract cell line (e.g., Reznikoff et al. 1989). Systems such as these may allow the dissection of the multiple stages in tumor development and the association of individual oncogenes with specific stages.

(2) The relationship of oncogene activation to proliferative capacity and growth factor dependencies of irradiated cells is not clear. Dividing cells may have a capability, different from nondividing cells, to respond to the radiation events that culminate in neoplastic transformation. A related unresolved issue is whether or not tumors result from cells whose normal process of differentiation has been aborted, from differentiated cells that have been induced to dedifferentiate, or from both processes.

(3) The effects of dose rate and radiation quality on oncogene expression are essentially unknown. A few data suggest that different oncogenes may be activated in the same tissue by a low- versus a high-LET radiation.

(4) Tissue and species differences in the activation of oncogenes by different radiations require clarification. This information, together with data on the mechanism(s) by which oncogene products exert their effects, may be useful in the problem of extrapolating from species to species.

(5) The mutations which have been scored in radiation-activated oncogenes are those which can be detected by the assays which are available. Improved detection, particularly of translocated DNA and other rearrangements, may permit the role(s) of other kinds of mutations to be identified.

(6) Attempts should be made to develop further test systems which will permit the identification of activated oncogenes within cells *in vitro* and in the same cells *in vivo*. It may be possible, thereby, to correlate changes *in vitro* with steps in tumorigenesis.

(7) Oncogenes which are identified by DNA-mediated transfection act dominantly. Other genes exist which cannot be detected by a transfection assay. In addition to dominantly acting oncogenes, there are genes which repress oncogenic expression (Klein and Klein 1985; Klein 1987). In some instances, the loss of a recessive gene is required for tumor development, for example, in familial retinoblastoma (Benedict et al. 1987).

(8) In addition to dominant and recessive oncogenes, a third category of genes is probably also involved in tumor development. These have been termed "modulators" (Klein and Klein 1985). The products of such genes are thought not to independently convert normal to transformed cells, but to modify the postinitiation stages of tumor development. Examples of such genes would include those controlling cell growth, differentiation, and proteolytic processes. The radiation-induced transformation of cells *in vitro* can be modulated by a variety of agents applied between the irradiation and the expression of the transformed phenotype (e.g., Gould et al. 1987). Both promoters (e.g., phorbol esters, Han et al. 1984) and antipromoters (e.g., vitamin E, retinoids, and protease inhibitors; Kennedy and Little 1978) are known to affect the expression of the transformed phenotype. Identification of the essential genes and the conditions under which they function will help in developing an understanding of the differences in transformation and tumor induction in different cells and tissues.

(9) Characteristic aberrations of the types produced by radiation, that is, translocations and deletions, are associated with specific human tumors, and often an activated oncogene (Yunis 1983; Rowley 1984), or an inactivated suppressor gene (e.g., Weinberg 1991), is located near a break point. Attempts should be made to characterize cytogenetic as well as genetic differences between transformed and nontransformed cells. Correlations should be sought also between the cytogenetic properties of transformed cells expressing the phenotype *in vitro* and those of the cells in tumors derived from them.

(10) Studies which are related to the foregoing and associated questions should be examined in reference to radiobiological parameters such as dose, dose protraction, and radiation quality to help identify those features which are important in radiation-induced neoplastic transformation.

Mutations

The likelihood that transformation constitutes a heritable change derived essentially from a somatic mutation points up the need to examine the mechanisms of mutagenesis, as discussed below.

(1) A number of approaches can be applied in the studies of mutagenesis. To characterize the molecular changes in a target gene, including an oncogene, amplification of the molecular signal may be required. To accomplish this objective, a number of factors should be considered.

(a) With increasing dose, the mutation frequency will increase and a larger proportion of the cells will contain multiple lesions and/or may be unable to survive the exposure.

(b) Generally, in order to identify a mutant some selection process is employed which facilitates the preferential growth of the altered cells. In the case of mutations in nonessential genes, a selective medium may allow the isolation and the clonal amplification of the cells which contain the mutation in a particular gene. Focus formation *in vitro*, the identification of emerging dysplastic or neoplastic cells, and tumor formation are examples of selection.

(c) The examination of genes in selected cells for mutations frequently is carried out by restriction-fragment analysis, oligonucleotide mis-match hybridization (if a particular mutant is sought), or by the more laborious process of recovering, cloning, and sequencing selected genes. Molecular characterization could be facilitated by the adaptation of newer methods for genomic sequencing (Church and Gilbert 1984) and the amplification of a gene segment by the polymerase chain reaction (Saiki et al. 1985). Whether or not such methods, or others (e.g., Lohman et al. 1987), are applicable to radiation-induced mutations has to be established in a given cell system in view of the diversity of expected mutations and particularly because the majority of the mutations are likely to be gross rearrangements or deletions. Future developments may be required to permit, for example, "walking" or "jumping" the genome from marker genes which have been identified as closely linked to the target genes of interest.

(2) The striking difference reported between the mutational spectra of spontaneous and radiation-induced mutations in the Chinese hamster *aprt* gene suggests the possibility of exploiting such a mutational fingerprint as a sensitive measure of the risks of low doses of radiation (Grosovsky et al. 1986; Grosovsky et al. 1988). This observation suggests that methods may need to be developed which allow the sequencing of a sufficiently large number of mutants to enable radiation-induced mutants to be characterized accurately, relative to those which appear spontaneously.

(3) In a detailed examination of radiation-induced changes in the coding sequences of a gene, a shuttle vector system could be employed providing that its spontaneous rate of mutation is low enough to permit radiation-induced mutations to be detected readily above background (e.g., Lebkowski et al. 1985). In general, only point mutations and small deletions can be measured in this way, but these do not comprise all of the kinds of lesions which are thought to give rise to a transformed phenotype. Still, in principle, measurements of the

dependence on radiobiological variables of the discrete molecular changes which can be detected with shuttle vectors would be an effective way of inquiring if such genetic changes could be responsible for radiation-induced neoplastic transformation.

(4) Mutational spectra may be of value in studying the mechanism. It will be useful to determine if the spectrum of mutations and, hence, of the mutational mechanism involved, is the same or different when mutants are induced by radiations of different qualities delivered at both high and low dose rates.

(5) The determination of mutational spectra in animals would provide a means to relate tissue differences and humoral controls to the development and expression of mutations *in vivo*. This approach could require the development of methods to recover cells from various tissues and to culture, amplify, and select *in vitro* the mutants of interest. At present, such a methodology has been worked out for lymphocytes and the *hprt* gene (Albertini et al. 1982), but, in principle, other tissues might be studied in a similar manner.

(6) Screening human populations is the most direct way of determining human risk. New techniques for detecting genetic changes in people are needed. Examples of innovative approaches may be found in the work of Albertini et al. (1982), in reference to the *hprt* gene, which is being applied by Seifert et al. (1987) to patients who have been examined by the techniques of nuclear medicine; the detection of allelic forms of glycophorin A (Jensen et al. 1987; Langlois et al. 1987); and in the detection of germ-line mutations by the examination of variant proteins in blood (Neel et al. 1988).

Induction Curves at High Dose Rate: Dependence on Position in the Cell Cycle and on Radiation Quality

Correlations should be sought between altered proto-oncogenes, or their expression, in transformed cells and the dependence of transformation on dose. Such an examination should be executed in a systematic way including high-dose-rate exposures to low-LET radiation, then to particle beams such as fission-spectrum and monoenergetic neutrons, and then to charged atomic nuclei of defined stopping powers.

A number of reports indicate that the transformation frequency due to x or gamma rays maximizes with dose (e.g., Terzaghi and Little 1976; Borek et al. 1978). This observation suggests that the dependence of susceptibility to transformation varies through the cell cycle because of the generally observed cycle-dependence of survival. Cells in an age-interval(s) that is sensitive to both killing and transformation would be progressively eliminated with increasing dose, with the result that the frequency *per surviving cell* would be expected to maximize (Elkind et al. 1988, 1991a, b). To understand the basis for possible changes in induction with changes in radiation parameters, it is of interest to know how transformation susceptibility and survival depend on a cell's position in its growth cycle, and if, and how, these dependencies vary with radiation quality. If structure is found in the patterns of the cell-cycle response for

transformation, as well as for survival, then it should be possible to interpret more readily the transformation induction curve of asynchronous, as well as noncycling cells. Correlations then could be sought between altered proto-oncogenes, periods during the cycle that are transformation sensitive, and the normal genetic events that influence cell growth. As noted earlier, a "window" of transformation sensitivity alone has been proposed (Rossi and Kellerer 1986; Brenner and Hall 1990). However, because of their sensitivity to killing in addition to their sensitivity to neoplastic transformation, the cell-cycle interval in and near mitosis has been identified as the sensitive window (Elkind 1991a, b).

The measurements alluded to above were in the context of cell transformation. Relative to somatic mutation, similar measurements of dependencies on radiation quality and cell-cycle position should be made. For this purpose, it would be useful to increase the sensitivity of the assay technique by using, for example, hybrid cells containing markers on nonessential chromosomes (Waldren et al. 1986), markers in a heterozygous configuration (Evans et al. 1986), or a transfected gene which may be mutated with high frequency compared to its constitutive analog (Thacker et al. 1983).

Dose Protraction: Repair/Misrepair Processes in Mutation and Neoplastic Transformation

Using the high-dose-rate induction curves obtained with a given cell system as a reference, the influence of dose protraction on neoplastic transformation should be examined. The radiations of immediate interest would be, for example, fission spectrum neutrons versus a low-LET radiation. Monoenergetic neutrons should be added in an effort to determine dependencies on neutron energy; the LET dependence could be examined further using track-segment irradiation with charged-particle beams; and the influence of the low-LET contamination of a neutron beam could be simulated by simultaneous irradiation with high-LET particles and a low-LET beam.

A distinction should be drawn between dose protraction due to continuous exposures at a low dose rate and multiple exposures at a high dose rate. Although in the limit, the latter method of protraction can converge on the former, in general, they are not equivalent. To illustrate, multiple-dose fractions using fraction intervals long compared to biochemical and/or biological characteristic times would very likely not introduce parasynchronous effects if the fraction size is small; in contrast, unless the dose rate is sufficiently low, parasynchrony could be encountered during continuous exposure, even if the conditions for cell growth are optimal. On the other hand, multiple doses separated by "large" intervals would not be sensitive to the time course of repair/misrepair processes if the latter are completed between dose fractions. Because of these differences, both methods of dose protraction should be considered including, for programmatic reasons at least, low dose rate exposures administered as a series of multiple-dose fractions. Other examples of differences that could exist between low-dose-rate and multiple high-dose-rate exposures could result from a dependence of the kind of mutations produced. For example, the relative frequencies of intragenic versus large extragenic changes like deletions could be dose-rate dependent.

As already noted, an objective of studies with protracted doses should be to seek molecular changes which correlate with the induction of cellular endpoints expressed by a given cell system, and then to examine additional systems to establish generalities. The molecular changes responsible for the altered phenotypes may depend upon the type of cells under study, as well as the radiobiological parameters in a given case.

Dose protraction should be examined using actively growing cells, as well as cells not in cycle. In a given case, the latter may more closely simulate the cells in tissue which may be the targets for tumor induction.

Interactive Effects of Different Radiations

Consideration should be given to the degree to which neutron radiation, for example, fission-spectrum neutrons, produces damage in cells that can interact with a low-LET radiation. For example, Ngo et al. (1977) showed that such was the case in cell killing and in the repair of sublethal damage when V79 Chinese hamster cells were irradiated sequentially with neutrons and with x rays. A similar observation was made by Han et al. (1984) using 10T½ mouse fibroblasts. Similar action with respect to other endpoints could help identify their genetic basis and their relevance to the issues of this program.

Chemical- Versus Radiation-Induced Changes

Many chemicals, which act directly or indirectly on DNA, produce radiation-like endpoints, and progress is being made in understanding their mechanisms of action. Although some chemicals are clastogenic, and therefore may be similar to radiation mechanistically, a number produce their effects via point changes in DNA. The combined action of chemical agents and radiation should be studied: first, because contrasting modes of action can help cross-illuminate the separate mechanisms involved; and second, because people are frequently exposed to both agents. To fruitfully employ mutagens and oncogens which require metabolic activation, cell systems should be used which have the required enzymatic machinery or in which the enzymes can be induced. It is also possible to add extracellularly an extract of microsomal enzymes derived, for example, from a liver of a suitable rodent. In principle, the combined action of physical and chemical agents offers another means of investigating the action of specific genes in inducing a loss of growth control because of the characteristically different kinds of genetic changes produced, and the likelihood that more than one oncogene may be involved in producing and/or expressing the transformed state.

Hormones are a special group of chemicals which may act as co-oncogens along with radiation (e.g., Kennedy and Weichselbaum 1981) or possibly as antioncogens. Such compounds can be effective *in vivo*, and, hence, this mode of action at a cellular level should be examined.

Modification of Rates of Neoplastic Transformation

Special classes of chemicals which can alter rates of neoplastic transformation are designated as *promoters* and *antipromoters*. These designations are operational, are based upon activities demonstrated *in vivo*, and reflect the ability of a chemical treatment to act after the completion of the initiating treatment, presumably by affecting an expression step in a multistep process. In contrast to chemicals which may be genotoxic by themselves, promoters and antipromoters generally demonstrate little if any independent biological activity, are considered to be ineffective if applied before treatment with an oncogen, and usually require an extended period of application after treatment with the inducing agent in order for an effect to be evident.

The mechanisms of action of promoters and antipromoters—for example, the phorbol esters and protease inhibitors, respectively, and the compounds which operationally have similar activities—are important in reference to how the expression of genetic changes may be modulated by the cell's metabolic machinery or by signal transduction via receptor sites on the plasma membrane. Also, chemicals which act in this way may be present in the environment and, consequently, could alter radiation-induced rates of transformation.

As in respect to radiobiological parameters, the actions of co-oncogens, antioncogens, promoters, and antipromoters offer additional opportunities to help to identify the genetic changes which are involved in radiation-induced neoplastic transformation.

Chapter VII

TISSUES AND ORGANS: DETERMINISTIC EFFECTS*

INTRODUCTION

In ICRP Publication 26 (ICRP 1977a), a distinction was made between "stochastic" and "nonstochastic" effects for purposes of radiation protection. In terms of an effect in an individual, a stochastic effect was defined as one for which the probability of occurrence, not the severity, is a function of dose, for example, the induction of cancer or hereditary change. In the 1977 recommendations, a nonstochastic effect was defined as one for which the severity of the effect in an individual would vary with dose. These effects are frequently associated with a large threshold. If enough cells in an organ or tissue are killed or prevented from reproducing and functioning normally, there will be a loss of organ function. In ICRP Publications 58 (ICRP 1989) and 60 (ICRP 1991) these "nonstochastic" effects are now called "deterministic" effects.

UNSCEAR (1982) reviewed the deterministic effects of radiation on normal tissues with the main emphasis on results in experimental animals. UNSCEAR found that an interpretive, rather than a comprehensive, treatment was necessary because the amount of information on the subject that had accumulated during the previous 20 years was so large. That treatment was facilitated by the significant advancement in the knowledge of the basic mechanisms of responses of cells and tissues to radiation exposure. The premise of UNSCEAR's review was that the deterministic response of a given tissue to radiation depends primarily on the level of killing of the component cells and that the degree of damage and its time of occurrence are related to the special characteristics of each tissue. It was indicated that the similarities between the observed effects in animals and human beings warranted a common summary of the subject matter, with qualifications, as necessary, to indicate major differences. The deterministic effects of radiation in tissues and organs are important considerations for radiation protection at moderate to high dose levels. Early effects in man after irradiation with high doses of low-LET radiation are summarized in UNSCEAR (1988). There is little experience on the incidence of deterministic effects in man after irradiation with neutrons, except in a few isolated accidents. The results of animal experiments are indispensable to obtain an indication concerning RBE values for deterministic effects in man.

Information on the dose effect relationship for the killing of normal cells in various tissues is essential to understanding the factors involved in the development of deterministic effects.

As UNSCEAR (1982) pointed out, a stochastic effect stems from injury in a single cell or a small number of cells and appears in an irradiated population as an hereditary effect or tumor, and a deterministic effect in a tissue or organ results from collective injury of many cells and appears as tissue or organ damage. In general, a deterministic effect requires a minimum

* The major part of the material in this report was prepared prior to June 1991.

significant dose (threshold dose) in order to be detected as physiologically significant, and the severity of the damage increases with increasing dose.

The time of appearance of the tissue damage is highly variable, ranging from a few hours or days to years after irradiation, depending on the type of effect, the characteristics of the particular tissue, the magnitude of the dose, and the dose rate.

The rate of development of injury depends on the life span of the mature functioning cells and the regeneration of proliferative cells. In rapid cell renewal systems, such as the bone marrow, intestinal epithelium, skin, and testis, the life spans of the mature differentiated cells range from a few days to a few weeks. In these hierarchical-type tissues, a stem cell compartment will produce new maturing cells (see Hendry and Fowler 1986). Slow renewal systems with a long life span of the functioning cells, such as the liver, kidney, bone, and the capillary endothelium, show relatively little proliferation under normal conditions. Although a stem cell compartment has not been identified for these tissues, damage to the parenchymal cells can induce accelerated proliferation of surviving cells. In nonrenewal systems, such as nerve cells and oocytes, cells are not replaced in normal adult organisms. Therefore, the rate of development of injury depends on the life span of the mature functioning cells, the proliferation rate of parenchymal cells, and the feedback signals, including growth factors, which may be generated in response to the injury.

The concept of a threshold dose has to be considered in relation to each tissue and each effect because the dose at which an effect is registered depends upon the sensitivity of the measuring technique. The loss of functional capacity of a given tissue actually may show a much higher threshold dose than that for the appearance of subtle cellular changes that may be detectable only by sophisticated technology. Therefore, it is important to distinguish the threshold of appearance of physiological changes which may have clear pathological connotations. Examples of responses for which a threshold dose exists are nonmalignant changes in the skin (erythema), cell depletion in the bone marrow causing hematological deficiencies, and gonadal damage leading to impairment of fertility. At the lower dose levels which often are encountered in radiation protection, failure of an organ to function is rare. In the absence of such physiologically evident effects, however, the introduction of more sensitive biological detection methods allows the quantitative determination of damage at the cellular level, including mitotic delay and damage to the reproductive capacity of individual cells. Furthermore, the postexposure time at which an effect may be detected will depend on the temporal course of the damage, which, in turn, will vary according to the extent to which the underlying injury is repaired or progresses with time after irradiation.

In ICRP Publication 41 (ICRP 1984), the deterministic biological and health effects of ionizing radiation were reviewed with particular reference to their implications for dose limits in radiation protection. The dose-response characteristics of stochastic and deterministic effects are illustrated in the ICRP figure reproduced here as Figure VII-1.

The killing of cells is a critical part of the pathogenesis of most, if not all, deterministic effects. In the context of the present discussion, the terms "cell killing" and "cell death" may refer to cell lysis, to the loss of a cell's reproductive (proliferative) capability, or to the impairment of its metabolic functions. The term "cell survival" is often used not merely to refer to metabolic integrity, but more often to the ability of a cell to proliferate indefinitely and to

form a colony of daughter cells. The mean radiation dose required to destroy a cell's reproductive capability is generally much less than that which is required to destroy its metabolic integrity (Elkind and Whitmore 1967; Rubin and Casarett 1968a; Hall 1978).

Figure VII-1. Characteristic differences in dose-effect curves between stochastic and deterministic effects. Deterministic effects vary in severity as well as frequency with the dose. Since the mechanisms of deterministic effects include cell killing and other stochastic effects which may in themselves be observable at incipient stages, delineation of the dose-response relation for any given type of deterministic effect depends on the stage and severity at which the effect is scored. The upper and lower figures illustrate how the frequency and severity of a deterministic effect, defined as a pathological condition, increase as a function of dose in a population of individuals of varying susceptibilities. The severity of the effect increases most steeply in those who are of greatest susceptibility (lower figure, curve a), reaching the threshold of clinical detectability as a pathological condition at a lower dose in this subgroup than in less susceptible subgroups (curves b and c). The range of doses over which the different subgroups cross the same threshold of severity is reflected in the upper curve, which shows the frequency of the pathological condition in the entire population, and which reaches 100% only at that dose which is sufficient to exceed the defined threshold of severity in all members of the population (ICRP 1984).

Although other aspects of pathogenic mechanisms may also be involved, the responses of tissues and organs *in vivo* usually have the general characteristics that have been shown in clonogenic cell-survival studies, including the sparing effect that usually results from reduction of dose rate in low-LET radiation exposure. The relationship of deterministic effects to magnitude of dose and to time after irradiation depends largely on cell reproduction, the post-irradiation growth kinetics of the critical target cells and their progeny, and the structure of the tissue or organ. Many of the more significant deterministic effects, that is, the "clinically significant" effects, may appear only after long latent or subclinical periods, depending upon the magnitude of the dose and the dose rate.

As organs consist of a variety of tissues and cells (parenchymal, vascular, connective, etc.) with different rates of proliferation and different levels of radiosensitivity, the expression of radiation injury tends to occur at different times in various cell population compartments among or within organs. Furthermore, under some conditions, the expression of radiation injury may be complicated by compensatory cellular or tissue reactions (hypertrophy, proliferation, and various homeostatic processes) which alter cell kinetics. Threshold doses for the impairment of function of various tissues and organs are dependent upon the various mechanisms of tissue and organ effects involved, even if there is no threshold dose for the injury or killing of target cells. Some of the aspects which influence threshold are: repopulation by proliferation of surviving cells; buffering of the consequences of damage in stem cell compartments by differentiating, maturing, and functional cells; compensatory changes in tissues to maintain the supply of differentiated cells or the functional capacity; functional reserve capacity of tissues; and dependence of effect on indirect as well as direct injury of cells or tissues, for example, tissue damage secondary to changes in supporting vascular and connective tissue elements, microcirculation, and various endocrine or other intertissue or interorgan reactions. These aspects may help explain why it takes a relatively large dose to produce a significant loss of tissue or organ function and why the thresholds depend on tissue types and functions.

GENERAL STATUS OF RADIOBIOLOGICAL INFORMATION

In reference to deterministic effects, there is now a vast, but far from complete, body of knowledge available about the molecular, cellular, histologic, biochemical, physiologic, pathologic, and actuarial effects of low-LET radiations in a wide variety of biological systems under various biological and physical conditions. Such information on deterministic effects of radiation in mammals has come largely from experimental studies on cells, tissues, organs, systems, and populations of many mammalian species and strains, especially small rodents, largely under conditions of radiation exposure of the whole or large segments of the body, at various levels of dose and dose rate.

During the past decade there has been appreciable progress in the interpretation of assays of normal tissue injury and of dose-response relationships based on these methods. Progress includes identification of target cell populations and their associated critical stromal tissues, which are involved in gross tissue damage. A number of assays of tissue injury and likely target cell populations are summarized in Table VII-1 (Hendry and Fowler 1986). The uncertainties

represented by the alternatives offered for likely target cell populations (separated by the slashes) suggest a need for further investigation.

Table VII-1. Assays for tissue injury and possible target cell populations.^a

Tissue	Assay of Tissue Injury	Likely Target Cell Population ^b
Skin	Erythema Deformities	Capillaries/epidermis Epithelial/stromal
Esophagus	Ulceration/fibrosis	Mucosa/stroma
Intestine	Denudation, protein leakage, LD _{50/7} ^c	Mucosal stem cells
Stomach	Emptying	Neurological/hormonal
Kidney and bladder	Weight, urinary enzymes Ureteral blockage Urinary frequency Bladder permeability Bladder capacity: edema/fibrosis	Tubule epithelium Epithelium Epithelium? Epithelium Muscle cells
Testis	Fertility Hormonal deficiencies	Stem cells Sertoli cells/Leydig cells
Central nervous system	EEG abnormalities White matter necrosis Nerve root necrosis Paralysis	Granular and subgranular zones Oligodendrocytes/vascular Schwann cells
Lung	LD _{50/180} , breathing rate Blood gases, gas transfer Lung weight, density	Type II cells/endothelial cells
Hemopoietic tissue	Blood and marrow counts, LD _{50/30}	Marrow stem cells

^a Summary from a more extended table of Hendry and Fowler (1986).

^b The terms following the slashes refer to alternative target cell populations.

^c The term LD_{50/7} denotes the determination of the dose that will lead to a 50% chance for mortality in an exposed population within 7 days after irradiation.

The information available on the deterministic effects of radiation in human beings is much more limited than that from experimental animals. This information has come largely from the studies of radiotherapy patients who received large fractionated doses to localized fields in the body and, to a considerable extent, from the studies of people who received large, brief exposures to the whole body; for example, Japanese A-bomb survivors. Studies of persons who were exposed accidentally or occupationally to radiation have also yielded some information (Lushbaugh et al. 1987).

Extensive inventories of radiation "tolerance doses" for normal human tissues and organs have been provided, based mainly on effects observed after fractionated low-LET photon irradiation regimens used in radiotherapy, that is, usually 20 to 35 fractions of the total doses of x rays or gamma rays over periods of 4 to 7 weeks (Rubin and Casarett 1968b, 1972; UNSCEAR 1982; ICRP 1984) (see Table VII-2). Such information that is relevant to dose-effect relationships for deterministic effects on human tissues and organs, coming as it does largely from experience with relatively high doses and dose rates, is delineated in terms of the doses and the regimens and conditions of radiation exposure that do or do not cause unacceptable deleterious side effects in radiation therapy. The scoring of such effects usually has been based on relatively gross or overt clinical manifestations. In this context, the term "tolerance dose" has been used in radiotherapy to indicate a dose associated with the absence or low frequency (e.g., 1-5%) of such overt side effects, without implying the absence of lesser, or subclinical, degrees of effect.

Such tolerance doses have been used as the bases for attempts to derive threshold doses for clinically significant effects (ICRP 1984). The term "clinically significant" has been used to denote a level of severity of effect which is not merely detectable, but is associated with considerable degrees of functional impairment or disease (ICRP 1984; NCRP 1987b). The NCRP pointed out that, if the deterministic endpoints of concern in radiation protection are limited to those that are physiologically significant or health-impairing, the threshold doses for those endpoints are high enough to permit selection of exposure limits that would avoid the occurrence of the specified effects. Updated information on the tolerance of normal tissues in man, with special emphasis on partial volume effects, was presented by a task force (Emami et al. 1991). It is quite interesting to note that the earlier observations of Rubin and Casarett (1972) on normal tissue tolerance are still valid for the majority of organs. Considerably lower dose values have now been reported for the TD 5/5 (probability of 5% complications within five years) and the TD 50/5 (probability of 50% complications within five years) for the lung and the heart. For irradiation of the total volume, these two dose levels now amount to 17.5 and 24.5 Gy, respectively, for the lungs and to 40 and 50 Gy, respectively, for the heart.

The extent to which workers are protected from the deterministic effects of occupational radiation exposure by the existing system of dose-equivalent limits has been assessed by considering these limits in relation to the aforementioned dose thresholds derived from tolerance doses for various tissues and organs (ICRP 1984). The recommendations of the ICRP (1991) are intended to prevent deterministic effects and to limit the occurrence of stochastic effects to an acceptable level.

Table VII-2. Estimates of approximate threshold doses for clinically detrimental deterministic effects in various tissues, based on responses of patients to conventionally fractionated therapeutic x or gamma irradiation.^a

Organ	Injury at 5 Years	Dose Causing Effect in 1-5% of Patients (Gy)	Dose Causing Effect in 25-50% of Patients (Gy)	Irradiation Field (Area)
Skin	Ulcer, severe fibrosis	55	70	100 cm ²
Oral mucosa	Ulcer, severe fibrosis	60	75	50 cm ²
Esophagus	Ulcer, stricture	60	75	75 cm ²
Stomach	Ulcer, perforation	45	50	100 cm ²
Intestine	Ulcer, stricture	45	65	100 cm ²
Colon	Ulcer, stricture	45	65	100 cm ²
Rectum	Ulcer, stricture	55	80	100 cm ²
Salivary glands	Xerostomia	50	70	50 cm ²
Liver	Liver failure, ascites	35	45	whole
Kidney	Nephrosclerosis	23	28	whole
Urinary bladder	Ulcer, contracture	60	80	whole
Ureters	Stricture, obstruction	75	100	5-10 cm
Testes	Permanent sterilization	5-15	20	whole
Ovary	Permanent sterilization	2-3	6-12	whole
Uterus	Necrosis, perforation	>100	>200	whole
Vagina	Ulcer, fistula	90	>100	5 cm
Breast, child	No development	10	15	5 cm ²
Breast, adult	Atrophy and necrosis	>50	>100	whole
Lung	Pneumonitis, fibrosis	40	60	lobe
Capillaries	Telangiectasis, sclerosis	50-60	70-100	—
Heart	Pericarditis, pancarditis	40	>100	whole
Bone, child	Arrested growth	20	30	10 cm ²
Bone, adult	Necrosis, fracture	60	150	10 cm ²
Cartilage, child	Arrested growth	10	30	whole
Cartilage, adult	Necrosis	60	100	whole
CNS (brain)	Necrosis	50	>60	whole
Spinal cord	Necrosis, transection	50	>60	5 cm ²
Eye	Panophthalmitis, hemorrhage	55	100	whole
Cornea	Keratitis	50	>60	whole
Lens	Cataract	5	12	whole
Ear (inner)	Deafness	>60		whole
Vestibulum auris	Meniere's disease	60	100	whole
Thyroid	Hypothyroidism	45	150	whole
Adrenal	Hypoadrenalism	>60		whole
Pituitary	Hypopituitarism	45	200-300	whole
Muscle, child	Hypoplasia	20-30	40-50	whole
Muscle, adult	Atrophy	>100		whole
Bone marrow	Hypoplasia	2	5.5	whole
Bone marrow	Hypoplasia	20	40-50	localized
Lymph nodes	Atrophy	35-45	>70	—
Lymphatics	Sclerosis	50	>80	—
Fetus	Death	2	4.5	whole

^a From Rubin and Casarett (1972). Also in UNSCEAR (1982) and ICRP (1984).

According to the 1991 recommendations of the Commission, deterministic effects will be prevented by applying an effective dose limit of 0.5 Sv/year to all tissues except the optic lens, for which a limit of 0.15 Sv/year was recommended. For irradiation of individual organs, the ICRP also recommended dose limits for stochastic effects by the introduction of weighting factors (ICRP 1991). For these stochastic effects, the occupational limit is 20 mSv/year, averaged over defined periods of 5 years. It was pointed out (ICRP 1984) that, in practice, tissues or organs were seldom, if ever, irradiated selectively in occupational exposure; and, consequently, their exposure is usually restricted to lower levels by the lower dose-equivalent limits for stochastic effects of the irradiated tissues or organs or for whole-body irradiation, as the case may be. Therefore, under usual conditions, the value of the cumulative lifetime dose-equivalent limit (25 Sv) for deterministic effects, based on 50 years of occupational irradiation, is, to a large extent, hypothetical.

Although detailed dose-effect curves for the frequency and severity of effects of short-term irradiation have been derived for many tissues and organs, comparable quantitative data for responses to long-term protracted irradiation are fragmentary (UNSCEAR 1982). Consequently, the dose thresholds for deterministic effects of occupational irradiation, and the index of harm (ICRP 1977b) attributable to such effects, cannot be quantified precisely. The tolerance doses (e.g., Table VII-2) are approximations at best, based on exposure regimens used in radiotherapy. It has been regarded as likely that reducing the doses by 20% or protracting them over a period of years would completely prevent severe deterministic effects in all exposed individuals (ICRP 1984). It has been noted (ICRP 1984) that the threshold values listed in Table VII-2 are based largely on observations in elderly patients. The values for younger and healthier persons might be higher, although it is also conceivable that they might be lower because of the longer life expectancy in which damage could be expressed. Notwithstanding these uncertainties, the ICRP concluded that an upward adjustment of the threshold values for the conditions of further protraction, as encountered in occupational exposure, would result in values well above 25 Sv for all tissues except the bone marrow, gonads, and optic lens. For these three tissues, the dose thresholds would be below 25 Sv, but they were still thought to be greater than the total doses that could accumulate from occupational exposure under the currently recommended occupational dose limits.

To summarize: in general, in the context of clinically significant effects, the experience over decades in the study of patients who received radiotherapy over a period of several weeks indicates that there are threshold doses when the endpoint is the functioning of tissues and organs. For x irradiation delivered via fractionated doses as in radiotherapy, the threshold doses for clinically significant damage vary depending upon the effect and the tissue, for example, from about 2-3 Gy for permanent impairment of ovarian gametogenesis to more than 100 Gy for atrophy of skeletal muscle. It may be inferred from available clinical and experimental data that the threshold doses for tissue tolerance in radiotherapy patients exposed to low-LET radiation are smaller than those for the much more protracted radiation exposure characteristic of occupational exposure. However, the magnitude of the difference is highly uncertain. For most deterministic effects, the relationship between severity and dose cannot yet be predicted for doses accumulated at low dose rates.

It has been argued (ICRP 1984) that in the cases of bone marrow and testes, protraction of the radiation dose over decades, as in occupational exposure, should minimize the effects because hemopoietic and spermatogenic cells are regenerated. In the case of the ovary and the optic lens, however, it is conceivable that annual exposures at the occupational dose-equivalent limit could result in cumulative doses approaching the dose thresholds for deterministic effects, but such exposure is highly improbable.

There is a substantial body of animal data, and some fragmentary human data, concerning the effects of low-LET radiation exposures of embryos and fetuses *in utero* at different stages of development and of immature animals *postpartum*. In addition to the high susceptibility of the embryo to radiation-induced aberrations of organogenesis, the embryo, fetus, and immature *postpartum* animals, in that order, are more susceptible than adults to radiation effects on development and growth and to the various deterministic effects of irradiation. Data on dose-response relationships for such effects, as influenced by age, are scanty, however. Certain effects on organogenesis and development, particularly in critical periods in the development of the central nervous system, have been observed following doses so small as to suggest the virtual lack of a practical threshold.

For radiation effects on the development of the human embryo-fetus, the period of highest sensitivity for the induction of developmental aberrations, especially in the central nervous system, lies between the 8th and 15th weeks after conception. The neuroblasts in the brain show the highest rate of proliferation during this period. On the basis of data on Japanese A-bomb survivors who were *in utero* at the time they were exposed, a linear dose-effect relationship has been reported for mental retardation in children (Otake and Schull 1984).

The deterministic effects produced by beta or gamma radiation from radionuclides deposited internally in the body are usually consistent in type and degree with those caused by comparable mean tissue doses of low-LET radiations from external sources at low dose rates. Auger electron cascades may have enhanced effectiveness, including high-LET-like characteristics, depending on the subcellular locations of the decaying radionuclides (Gaulden 1983; Lancet 1985; CEIR 1986). The tissues affected by irradiation from a given internal radionuclide depend on the particular distribution of that nuclide in the body. The amount of injury depends on the characteristics of the radiation and on the temporal and spatial distribution of the energy absorbed.

Models relating the temporal and spatial distribution of absorbed radiation doses from an internally deposited radionuclide to that of fractionated or protracted irradiation from external sources have not yet been fully explored on the basis of equal effects. There are also uncertainties in determining the microdistribution of the energy delivered to the biological targets within the cells and how they affect the assignment of precise values of relative biological effectiveness to short-ranged radiations from radionuclides.

RELATIVE BIOLOGICAL EFFECTIVENESS OF FAST NEUTRONS

Although the microdosimetric spectra for various neutron beams extend over an appreciable range of LET values (see Chapter IV), fast neutrons are generally described as high-

LET radiation. A conclusion from studies on a number of *in vivo* and *in vitro* biological systems is that the higher RBE values are observed for neutron energies in the vicinity of 0.5 to 1.0 MeV, with a monotonic decrease of RBE as the neutron energy increases (see Figure VII-2). Neutrons with these energies result from the fission process or are produced through the p+T reaction. Neutrons of 0.024 MeV energy have been found to have RBE values similar to those for fission neutrons (Morgan et al. 1988; Lloyd et al. 1988). The RBE values for neutron energies in excess of 10 MeV are of importance in radiotherapy applications.

Figure VII-2. Relative biological effectiveness as a function of neutron energy for 50% growth inhibition of *Vicia faba* seedlings (curve 1), 20% cell reproductive death in cultured human cells (curve 2), and 99% cell reproductive death in Chinese hamster cells (curve 3)(ICRU 1977).

As noted in earlier chapters, the effectiveness of neutrons relative to that of low-LET radiation increases with the fractionation of the dose. Because cellular recovery (repair) is generally relatively less effective after neutron irradiation, the doses of neutrons that could be tolerated if given over a long period of time are not thought to be much greater than those for the same radiation given in an acute exposure. This effect is observable for tissues in which postirradiation repopulation of cells is prominent, as well as in slowly repopulating tissues.

Due to the reduced influence of repair processes for high-LET radiation, the relative biological effectiveness of fast neutrons increases with decreasing dose rate or decreasing dose per fraction. This is exemplified in Figure VII-3, which shows RBE values for deterministic effects of 15 MeV neutrons, produced by the d+T reaction, on some normal tissues and experimental tumors in animals (Broerse and Barendsen 1973). A similar trend (with higher RBE values) has been observed for cyclotron neutrons, produced by the d+T reaction, with a mean energy of 7 MeV (Field and Hornsey 1979).

Figure VII-3. The relationship between RBE and dose of 15 MeV neutrons for effects on different normal tissues and two experimental tumors (Broerse and Barendsen 1973).

A substantial body of data on the relative biological effectiveness of neutrons for normal tissue response in experimental animals has been obtained by applying the assays mentioned in Table VII-1. Examples of RBE values for deterministic effects in various tissues are summarized in Table VII-3. In view of the dependence of RBE on neutron energy, separate values are mentioned for three neutron energies. In general, the RBE values were derived with reference to orthovoltage x rays at dose levels around 5 Gy of neutrons. Contrary to the RBE for stochastic effects, the choice of the reference radiation does not influence the RBE for deterministic effects to a large extent. As stated by the ICRU (1986), at absorbed doses in excess of about 1 Gy the biological effectiveness of electromagnetic radiations does not change by more than 20% over an energy range from 10^4 to 10^8 eV. Complementary information on the RBE for deterministic effects and maximum RBE values derived from the mathematical linear-quadratic formalism can be found in ICRP Publication 58 (1989). Depending on the type of tissue, the maximum RBE values generally vary between 4 and 8 for neutrons with energies of 1-5 MeV, with the exception of an RBE of 12 for kidney glomerular or tubular damage (Joiner and Johns 1988).

In connection with Figure VII-3 and Table VII-3, the following comments should be made. The studies of Bradley et al. (1981b) and Zook et al. (1981, 1983) were performed with ranges of doses (10 to 34 Gy of 15 MeV neutrons) delivered in a fractionated fashion, representing typical radiotherapy regimens. Because these data pertain to relatively low neutron doses per fraction, the RBE values are higher than other data that have been quoted. At low dose levels a relatively high RBE value has been observed for effects on the spinal cord (Van der Kogel 1979) which classifies this organ as a critical, dose-limiting, normal tissue in radiotherapy applications.

A limited number of studies have been performed on teratogenic effects of neutron irradiation in animals. Molls et al. (1981) investigated the production of micronuclei with 7 MeV neutrons at the two cell stage and obtained values of the relative biological effectiveness of 7.4, 5.5 and 1.8 at absorbed dose values of 0.05, 0.1 and 1 Gy, respectively.

Over the neutron dose range of 0.12 to 1 Gy, Streffer and Molls (1987) reported RBE values from 9.0 to 3.5 for the reduction in blastocyst formation in mouse embryos. Pampfer and Streffer (1988) studied malformations after irradiation of mouse zygotes and reported RBE values, for 7 MeV neutrons, of between 2.0 and 2.8 as the reference x-ray dose increased from 0.5 to 2 Gy. In his studies on postimplantation defects in mice, Konermann (1987) derived for the same neutron energy, RBE values between 1.6 and 2.9 for skeletal alteration and of approximately 2 and 4 for decrease in body and brain growth, respectively.

The lens of the eye has a relatively high radiosensitivity. According to the general principle the RBE increases with decreasing dose per fraction with an RBE value of up to 20 reported for 0.01 Gy per fraction (Di Paolo et al. 1978). Bateman et al. (1972) obtained even higher RBE values for the induction of microscopic changes in opacity in the mouse lens after long observation periods. At high doses these opacities developed within months, progressed rapidly, and eventually clouded the lens completely.

Table VII-3. RBE values for normal tissue injury in mammals for three neutron energies and neutron absorbed dose values in the range of 3-7 Gy.

Tissue and Species	Mean Neutron Energy			Reference Radiation	Literature Source
	1 MeV	7MeV	15 MeV		
Skin, mouse, pig		2.2		gamma rays	Field & Hornsey (1979)
Skin, rat		1.6		x rays	Broerse & Barendsen (1973)
Intestine, LD _{50/5} , mouse	3	2.8	1.4	x rays	Broerse & Barendsen (1973)
Intestine, crypt survival, mouse			2.1	gamma rays	Zywietz et al. (1979)
Intestine, pathology, dog			4.5	gamma rays	Zook et al. (1983)
Kidney, mouse	2.4			x rays	Stewart et al. (1984)
Kidney, pig		1.2		x rays	Hopewell (1987)
Bladder, mouse	3.2			electrons	Stewart et al. (1986)
Testis, mouse	5.7	3.0		x rays	Gasinska et al. (1987)
Ovary, mouse	1.3		1.0	gamma rays	Straume et al. (1987)
Central nervous system, rat		2.4	2.0	x rays	Hornsey et al. (1981)
Central nervous system, dog			3 ^a	gamma rays	Zook et al. (1981)
Lung, LD _{50/120} , mouse		1.6		x rays	Field et al. (1976)
Xe clearance, pig			1.3	x rays	Hopewell (1987)
Xe clearance, dog			4.4	gamma rays	Bradley et al. (1981b)
Hemopoietic tissue					
LD _{50/30} , mouse	1.9	1.5	1.1	x rays	Broerse & Barendsen (1973)
LD _{50/30} , monkey	2			x rays	Broerse et al. (1978b)
LD _{50/30} , dog	2.3			gamma rays	MacVittie et al. (1984)
LD _{50/30} , dog	1.0 ^b			x rays	Ainsworth et al. (1965)
LD _{50/30} , dog			1.0 ^b	x rays	Earle et al. (1971)

^a Fractionated irradiations with four fractions per week for 6 weeks.

^b 1 MV x rays.

Human oocytes are radiosensitive, but many can be lost without affecting fertility. No deleterious effects on reproductive function appear after doses to the ovary up to about 0.6 Gy of low-LET radiation. The murine oocyte is much more sensitive than the human with an LD₅₀ for oocyte killing of 0.06 to 0.12 Gy (Dobson et al. 1987).

The hemopoietic system is among the most radiosensitive tissues in animals and in man. Experiments in primates are of relevance since the response to radiation of primate species does not seem to be significantly different from that of man. After total body irradiation, the bone marrow syndrome would be expected as the first detectable effect to be seen with increasing dose levels. The survival results for Rhesus monkeys after irradiation with 300 kV x rays and fission neutrons are shown in Figure VII-4.

Figure VII-4. Survival of Rhesus monkeys within 30 days after total body irradiation with fission neutrons and 300 kV x rays without any treatment (open symbols) or followed by an autologous bone marrow transplantation (closed symbols). At each dose level the number of surviving animals and the total number irradiated are given. The dashed lines were based on earlier dosimetry for the x irradiations.

Without any supportive care, the LD₅₀ values for x- and neutron-irradiated monkeys are 5 and 2.6 Gy, respectively, resulting in an RBE of approximately 2 for the occurrence of the hemopoietic syndrome (Broerse et al. 1978b). This estimate is based on the average total absorbed dose (neutrons plus photons) in the case of the irradiation with fission neutrons. The contribution from photons to the total absorbed dose in the neutron-irradiated monkeys is approximately 30%. Consequently, the relative biological effectiveness for the occurrence of the bone marrow syndrome due to the neutron component alone is greater than 2. Photon production of a neutron beam should be accounted for in the case of neutron irradiations of relatively large animals.

When monkeys received their own bone marrow after irradiation (autologous transplantation), the LD₅₀ values were substantially increased. Protection by autologous bone marrow transplantation was evident for doses up to 8 Gy of x rays and 4.4 Gy of fission neutrons. For higher doses, bone marrow grafting was not effective because the majority of the animals died within seven days apparently due to severe damage to the small and large intestines (Broerse et al. 1978b).

The experiments with the Rhesus monkeys were performed under certain standardized conditions (Broerse et al. 1978b). Special provisions were made to achieve a dose distribution as homogeneous as possible. In the case of an inhomogeneous irradiation, the central dose required to produce the same biological effect would be considerably higher than the dose for uniform irradiation. If the ratio of the maximum to minimum absorbed doses over a monkey's body were increased from 1.05 (essentially homogeneous) to factors of 2, 4, and 8, the corresponding LD₅₀ values expressed as midline doses for the occurrence of the bone marrow syndrome can be estimated to increase from 5 Gy to 6 Gy, 7.8 Gy and 10.4 Gy, respectively (Zoetelief et al. 1984). For heterogeneous irradiation, the actual survival of different cellular components of the hemopoietic system determines the chance of survival and the introduction of specific curative or supportive methods (Fliedner et al. 1984).

Only a few comparisons have been reported on RBE values for normal tissue effects in various animal species and man. For early effects on skin, closely similar RBE values have been observed for irradiation of mice, rats, pigs and man exposed to fast neutrons with a mean energy of 7.5 MeV (Field and Hornsey 1979). Also, no strong species dependence was found in experimental studies on injury to the spinal cord in mice, rats, monkeys, and man (Hornsey et al. 1981).

Follow-up studies of deterministic effects in persons who received localized neutron radiotherapy have been increasing, but quantitative data are still fragmentary and markedly limited in scope. The optic lens is one of the few human tissues for which values of relative biological effectiveness for neutrons have been reported. In radiotherapy patients treated with 7.5 MeV neutrons delivered in 12 fractions over a period of one month, the threshold for impaired vision due to cataracts appeared to be about 3 to 5 Gy (Roth et al. 1976). For low-LET radiation fractionated over comparable time intervals, a dose in excess of 8 Gy was required to produce a vision-impairing cataract. From these data, one can infer that at such high dose levels the relative biological effectiveness of 7.5 MeV neutrons for induction of cataracts may be in the vicinity of 2 to 3.

CHEMICAL MODIFIERS OF DETERMINISTIC EFFECTS

Most studies on chemical radiation protection have been done using low-LET photon radiation. There is substantial evidence that significant chemical protection against gamma radiation is possible (Livesey and Reed 1987). Historically, development of radioprotective agents has been dominated by the study of sulfhydryl compounds (see, e.g., Adams 1970; Foye 1981), particularly the aminothiols and the more effective phosphorothioates (Davidson et al. 1980; Brown et al. 1988).

However, chemical protection against high-LET exposure is not as effective, most likely because it is more difficult to protect against the direct effects of radiation in contrast to indirect effects (mediated by reactive oxygen species). Studies on the differences in protection against photon and neutron irradiation can provide insights into the mechanisms of chemical damage to biomolecules by neutron exposure. Ainsworth et al. (1989) reviewed the early and late murine tissue responses to single and fractionated low doses of heavy charged particles, fission spectrum neutrons, and gamma rays, and the potential for chemical radioprotection against these injuries. It is encouraging that, although protection against neutrons is lower compared to gamma irradiation, significant protection has been obtained with phosphorothioates using 30-day (hematopoietic damage) and 6-day (gastrointestinal damage) mouse survival and survival of intestinal cells (Rasey et al. 1984; Sigdestad et al. 1986; Steel et al. 1987; Hanson and Grdina 1991) as endpoints. Maximum dose reduction factors (DRFs, the ratio of the radiation dose survived by 50% of drug-treated animals to the radiation dose survived by 50% of nontreated animals) against neutrons using the 30-day lethality endpoint were approximately 1.2-1.4 (Rasey et al. 1984; Steel et al. 1987), compared to approximately 2 for a similar dose of WR-2721 using photon irradiation (Yuhás and Storer 1969; Weiss et al. 1990).

Studies on radioprotection against neutrons indicate that the degree of radiation protection by sulfhydryl compounds is inversely proportional to the LET (Bird 1980; Steel et al. 1987). Jordan et al. (1961) and Thomson (1962) studied aminoethylisothiuronium (AET) and nitriles as protectors against neutrons. Considerable protection was reported by Sigdestad et al. (1986) with two phosphorothioate compounds using three neutron sources of average energies of 0.85 MeV, 1.2 MeV, and 25 MeV. WR-151327 protected mice against the lethal effects (6-day survival) of fission neutrons to a greater degree than against the effects of high energy neutron sources. However, there was no difference in the protection of crypt cell survival. Radioprotection by these two agents was also confirmed by Steel et al. (1987). A relatively low dose of WR-151327 (200 mg/kg) provided significant protection (180-day survival) against a 5.66 Gy mixed field ($n/\gamma = 1$) irradiation (Ledney et al. 1994).

Although phosphorothioates consistently produce high levels of protection, they suffer from one major drawback: high levels of protection are achieved only at doses accompanied by unacceptable side effects. Clinical studies of patients receiving this drug have shown that its administration is accompanied by a variety of side effects that include nausea, vomiting, and hypotension (Kligerman et al. 1984).

Although the most effective chemical protectors that can be given shortly before radiation exposure should be compounds that are effective radical scavengers or can efficiently donate hydrogen for repair of damaged biomolecules, drugs can also interfere with "slower reactions"

involved in radiation damage or in the interaction of radiation-induced reactive oxygen species with normal physiological oxidative reactions. This idea is validated by the ability of some antioxidant compounds (e.g., selenomethionine [Weiss et al. 1992], Vitamin E [Srinivasan and Weiss 1992], and Vitamin A [Seifter et al. 1984]) to protect mice against lethality even when the compounds are administered after gamma irradiation. Very little data exists on survival after neutron irradiation when chemical agents are administered postirradiation.

Biologic response modifiers containing trehalose and 2 mycolic acid residues (TDM, trehalose dimycolate) increased survival of mice given an approximate equal dose of fission neutrons and gamma rays. The DRFs for 30-day survival were about 1.1 when given 24 hours before irradiation or 1 hour after irradiation (McChesney et al. 1990).

THERAPY AFTER FISSION NEUTRON RADIATION EXPOSURE

Therapeutic modulation of the dose-response relationship over the hemopoietic syndrome can be accomplished with aggressive clinical support and/or the use of recombinant cytokines specific for hemopoietic progenitor cells (Schuening et al. 1989; MacVittie et al. 1984; Patchen et al. 1990; Tanikawa et al. 1989; Kobayashi et al. 1987). The results using cytokines concentrate on animals exposed to low-LET ionizing radiation. There are no reports on the use of cytokines in treating animals exposed to fission neutron radiation. MacVittie et al. (1991) have shown that a clinical support regimen consisting of antibiotics, fluids, and fresh platelets can increase survival in mixed fission neutron/gamma irradiated canines from 153 cGy to 185 cGy (DMF = 1.21). Such clinical support must be the base upon which new therapeutic regimens using cytokines can be evaluated in fission neutron-irradiated animals. An aggressive clinical support regimen may be particularly effective in neutron-irradiated animals because of the increased biological effectiveness for gastrointestinal damage in the canine and nonhuman primate. The higher RBE for gastrointestinal effects will decrease the dose range over which "hemopoietic" cytokines will be effective. MacVittie et al. (1984) have shown that gastrointestinal damage encroaches upon the hemopoietic syndrome range and will decrease survival time in spite of marrow engraftment. Those data indicate that clinical support for gastrointestinal damage must be included in prospective cytokine therapy protocols.

When individuals are likely to be exposed to a high total dose of protracted radiation, combined treatments involving agents that work by different mechanisms may be the strategy of choice. Significant increases in protection by phosphorothioates can be achieved by the addition of prostaglandins (Hanson 1987) and other biological response modifiers (Patchen et al. 1988). Combinations of agents may be effective against neutron irradiation (Hanson and Grdina 1991), as they are against gamma irradiation (Weiss et al. 1990). Misoprostol, a prostaglandin analog, administered alone or in combination with WR-2721 increased survival following exposure to fission neutrons (Hanson and Grdina 1991).

RESEARCH NEEDS

A substantial amount of research is needed on the effectiveness and relative biological effectiveness of irradiation with neutrons of various energies, with respect to dose-response relationships for deterministic pathologic and physiologic effects in the various normal tissues and organs of mammals, including man. The practical purposes to be served by this research are needed improvements in: (1) radiation protection from the effects of neutrons and other high-LET radiations over a wide range of doses and different applications, including space flights; (2) the effectiveness and safety in the use of neutrons and other high-LET radiations in cancer therapy; and (3) the knowledge of the fundamental mechanisms in radiation pathology.

Categories of needed research on the deterministic effects described generally above are outlined below.

(1) Dependence of the response of normal tissues in animals on neutron energy, including energies in excess of 15 MeV. Neutron beams with these energies are produced by high-energy cyclotrons and linear accelerators presently used for fast neutron radiotherapy. In view of the ongoing explorative studies on the possible applications of boron neutron capture therapy, investigations on the deterministic effects of neutrons in the energy range of 10 to 100 keV are also relevant.

(2) Dependence of the response of normal tissues on dose rate (fractionation/protraction), including long-term irradiation.

(3) Comparative mammalian studies, including larger laboratory animals (e.g., dogs, monkeys) to facilitate the extrapolation of principles to man and to enable the investigation of the effects of localized (partial-body) irradiation on organs.

(4) Relationships between normal cell kinetic parameters and the timing and extent of expression of the injury in the form of damage in various tissues and organs.

(5) Increased effort and new methods to derive quantitative data from studies of radiotherapy patients and other human subjects exposed to considerable neutron irradiation.

(6) Effects on the embryo and fetus irradiated *in utero*, and the dependence of the response to irradiation after birth as a function of animal age or stage of development.

(7) Development of prophylactic and therapeutic regimens for persons exposed in criticality accidents or military scenarios.

Chapter VIII

STOCHASTIC EFFECTS*

INTRODUCTION

The late radiation effects of main concern in radiation protection are tumorigenesis and hereditary effects. The central issue is to quantify the risks of inducing tumors of different types in various tissues after low-level neutron exposure and to quantify the heritable genetic damage. For both types of effects, there is a great paucity of quantitative data on humans after exposure to neutrons at both low and high dose levels because neutron fields are rare and exposure levels have been carefully limited. Consequently, the human data necessary for direct estimation of risk are not available and are unlikely ever to be sufficiently extensive. Hence, methods of indirect estimation of risk are essential.

The various aspects of carcinogenesis after exposure to high-LET radiation will be discussed with special emphasis on tumor induction after high-dose irradiation, analysis of tumor incidence data, life span shortening, the nature of dose-response relationships, relative biological effectiveness (RBE), and the effects of dose fractionation or protraction. For further details on the RBE of radiations of different quality for stochastic effects, the reader is referred to NCRP Report No. 104 (NCRP 1990).

In the past four or five decades virtually every convenient genetic test system has been utilized to predict radiation-induced hereditary effects in man. A number of observations concerning the genetic effects of neutrons on plant systems, insects, and rodents will be summarized. Up to the present, genetic experiments on mice form the main basis for genetic risk estimates for exposure of man to ionizing radiation.

RADIATION CARCINOGENESIS

Risk Factors at High Dose Levels

The radiation quality, which may be quantitatively expressed as linear energy transfer (LET), influences the detrimental effects. Radiations of differing LET have a different RBE for different biological endpoints, including tumor induction.

As concluded by UNSCEAR (1988, paragraph 201):

Lifetime cancer experience is not yet available for any of the large epidemiological studies. Therefore, to project the overall cancer risk for an exposed population, it is necessary to use models that extrapolate over

* The major part of the material in this report was prepared prior to June 1991.

time data based on only a limited period of the lives of the individuals. Two such projection models have received particular attention: (a) the additive model which postulates that the annual excess risk arises after a period of latency and then remains constant and (b) the multiplicative model in which the time distribution of the excess risk follows the same pattern as the time distribution of natural cancers, i.e., the excess (after latency) is given by a constant factor applied to the age dependent incidence of natural cancers in the population.

Cancer may be induced by radiation in nearly all tissues of the human body. However, it has been shown that tissues and organs can vary considerably in their sensitivity to the induction of cancer by radiation. The reassessment of the radiation dosimetry for the survivors after the atomic bomb explosions at Hiroshima and Nagasaki (RERF 1987) have resulted in increased risk factors for mortality due to radiation-induced tumors in different organs (Preston and Pierce 1987; Shimizu et al. 1989) in comparison with earlier recommendations (ICRP 1977a): projections of the lifetime probability of fatal cancers for 1 Gy whole-body, low-LET radiation yield values between 4×10^{-2} and 5×10^{-2} using the additive model and between 7×10^{-2} and 11×10^{-2} using the multiplicative model. A number of tissues with high sensitivity for cancer are summarized in Table VIII-1.

Table VIII-1. Sensitivity of various tissues to oncogenic influence and related mortality risk factors at low doses and low dose rates (ICRP 1991).

Tissue or Organ	Spontaneous Incidence of Cancer	Relative Sensitivity for Radiation Carcinogenesis	Mortality Risk Factor (Sv^{-1})
Female breast	+++	+	2×10^{-3}
Lung	+++	++	8.5×10^{-3}
Stomach	+	+++	11×10^{-3}
Colon	++	++	8.5×10^{-3}
Bone marrow	+	++	5×10^{-3}

The mortality risk factors as stated by the ICRP (1991) are extrapolated from human epidemiological data obtained at high doses (in excess of 0.3 Gy) to the domain of low doses and low dose rates using the multiplicative model. These values apply to a nominal population of equal numbers of men and women. The fatal probability coefficients for colon and stomach cancer suggested in 1991 are considerably larger than the 1977 recommendations, whereas the new risk factor for the breast is somewhat lower than the earlier estimate. These unexpected deviations have to be attributed to the use of the multiplicative model applied only to the

Japanese survivor data. It should be noted that the spontaneous incidence of mammary cancer in the western countries is higher than in Japan. For stomach cancer in males, a higher spontaneous incidence is observed in Japan than in western countries.

The tumorigenic effects of ionizing radiation can be modified when the time course of the irradiation is changed by fractionation or protraction of the dose. The introduction of a reduction factor varying between 1 and 3 to convert the risk at high doses to that at low doses and low dose rates is still under discussion. UNSCEAR (1988) followed the suggestion of NCRP (1980) that the appropriate value to be applied to total risk for low dose and low dose rate should lie between 2 and 10. It was further realized that the reduction factor will vary widely with individual tumor types. On the basis of a review of available data on cancer induction in experimental animals, Stather et al. (1988) judged that a reduction factor of 3 would be most appropriate for estimating the carcinogenic risks in most tissues, whereas a reduction factor of 2 was suggested for the breast. Studies on tumor induction in experimental animals can provide pertinent information concerning the extrapolation procedures.

For doses below 0.3 Gy of low-LET radiation, such as gamma rays, epidemiological data have not provided reliable evidence for the carcinogenic effect of radiation in man. Information on the effects of high-LET radiation was derived earlier from a follow-up of the Japanese survivors employing the Tentative 1965 Doses (T65D values). With the new dosimetry system (DS86), the neutron dose values in Hiroshima and Nagasaki are very much reduced with respect to the earlier estimations. Nevertheless, Shimizu et al. (1989) estimated some RBE values of neutrons relative to gamma rays from a comparison of the cancer induction results in the two cities. The RBE for leukemia might be in the range from 20 to 30 and for some other cancers might be in excess of 40. However, for cancers of the stomach, lung, and female breast, the RBE values are less than unity. The uncertainties in the DS86 dosimetry have been estimated to be about 40%, and for the assessment of the RBE values the uncertainties will exceed these limits. Therefore, it is essential to perform additional studies on experimental animals with the objectives to assess the nature of dose-response relationships, to determine the RBE of radiations of different quality, and to investigate the influence of fractionation or protraction of the dose on tumor development.

As a consequence of the relatively rapid relaxation of international tensions between the former Soviet Union and the West, information regarding new human data bases for both chronic and acute exposures to ionizing radiations, including neutrons, has emerged in recent years (Nikipelov et al. 1990; Medvedev 1991). Nikipelov et al. (1990) described the chronic exposures to the individuals involved in the development of the Soviet nuclear weapons program at Chelyabinsk during the period from 1948 through 1974. In the early years, the average annual exposures to this population were as high as 1.1 Sv, based on film badge records. Because of the nature of the work performed at these installations, it is likely that the exposures included a meaningful neutron component, which may or may not have been noted in the dosimetry records. Nikipelov et al. (1990) briefly described the observed health effects in this population including cancer mortality rates. Future analyses of these data are required to determine if the available information can be used to perform human risk assessments for neutrons.

Tumor Induction After High-Dose Irradiation

Studies in larger animals are of importance since the response of monkeys, pigs, or dogs to radiation has similarities to the effects in man. Studies in which the animals are kept under long-term observation after neutron irradiation are very limited.

Deeg et al. (1983) exposed dogs to ^{60}Co gamma radiation with a single dose of 6.1 to 11.4 Gy or fractionated irradiations with total doses of 7.6 to 21.3 Gy. The exposed dogs had an estimated fivefold higher risk of developing any malignancy than did the control dogs.

Partial body irradiations of beagle dogs were performed by Bradley et al. (1981a) to investigate early and late effects on the canine brain, spinal cord, and lung when clinical fractionation schedules are applied in radiotherapy. The total doses used were 10 to 26 Gy of $\text{d}(35)+\text{Be}$ neutrons with a mean energy of 15 MeV and 35 to 79 Gy of ^{60}Co gamma rays. For the period of observation of the group of 46 dogs irradiated with collimated neutron beams, there was a 15% incidence of neoplasms within the radiation field (in seven dogs) and zero incidence at sites outside the radiation field and in the controls.

The risks of total body irradiation with large doses of x rays and fission neutrons were investigated by keeping the long-term surviving monkeys from an experiment on acute effects (Broerse et al. 1978b) under continuous observation. Rhesus monkeys of a comparable age distribution were maintained under identical conditions of housing and nutrition to serve as a control group and were inspected frequently. In some cases, the animals were killed when moribund; a complete necropsy was performed as soon as possible after the death of an animal. The average absorbed doses in the monkeys were equal to 6.7 Gy and 3.4 Gy following irradiation with x rays and fission neutrons, respectively. The data on longevity and tumor incidence in the irradiated monkeys and in the control group are summarized in Figures VIII-1 and VIII-2, respectively.

The latent periods for neoplastic diseases varied between 7.5 and 15.5 years for x-irradiated animals and between 4 and 18 years after neutron irradiation. Nine out of 20 monkeys in the x-irradiated group and 7 out of 9 monkeys in the neutron group died with malignant tumors (Broerse et al. 1991). In the group of 21 untreated control monkeys, two animals died at the age of 23 years with uterine cervical cancer and gastric cancer, respectively. Although the total number of animals included in the study was rather limited, it is noteworthy that the mortality from cancer (two out of seven) in the group of unirradiated monkeys resembles the situation in the aging human population.

The tumors observed included glomus tumors, osteosarcomas, renal adenocarcinomas, thyroid follicular carcinomas, and tumors of the central nervous system. In a number of cases the observations at necropsy revealed multiple tumors in the same animal (Broerse et al. 1989). The induction of malignant glomus tumors is of interest because this tumor type is rarely observed in man and has not occurred in the unirradiated animals. The incidence of intracranial tumors is in accordance with the results of Yochmowitz et al. (1985), who performed a lifetime study of monkeys after single total body exposure to monoenergetic protons with doses between 1 and 8 Gy.

As indicated in Figures VIII-1 and VIII-2, the results can be analyzed in terms of the number of animals developing tumors per group as a function of the total observation period for

Figure VIII-1. Tumor incidence and postirradiation observation periods for long-term surviving Rhesus monkeys after whole body irradiation and autologous bone marrow transplantation. The dashed portion of the lines indicate the approximate age of the monkeys before entering the colony. Lines ending in cross bars signify death, and arrow heads indicate that the monkeys are still alive. (Broerse et al. 1991)

Figure VIII-2. Tumor incidence and observation periods in untreated Rhesus monkeys which served as an age-matched control group for the experiment on radiation carcinogenesis. (Broerse et al. 1991)

the entire group and the average absorbed dose. Thus, in the neutron irradiated group, seven monkeys developed malignant tumors in a total observation period of 90 monkey-years. For the x-irradiated group this number is equal to 10 per 237 monkey-years and for the control group 2 per 460 monkey-years. Risk factors for tumor induction can be derived as $(10/237 - 2/460) \times 6.7^{-1} = 57 \times 10^{-4} \text{ year}^{-1} \text{ Gy}^{-1}$ for x rays and $(7/90 - 2/460) \times 3.4^{-1} = 216 \times 10^{-4} \text{ year}^{-1} \text{ Gy}^{-1}$ for fission neutrons, resulting in an RBE of 4. It should be realized that these risk factors are calculated on the assumption of a linear dependency of the dose-effect relationship; are derived from tumor incidence data obtained at relatively high doses; and pertain to risk factors per monkey-year. Furthermore, this approach does not take into account the time-dependence of the tumor appearance. If one applies the concept of cumulative tumor rate (Broerse et al. 1991), the cumulative hazards are 0.64 and 0.18 for neutrons and x rays, respectively, at 10 years postirradiation. On the basis of such a calculation, the RBE for tumor induction in Rhesus monkeys with reference to x rays would be equal to $0.64 \times 3.4^{-1} / (0.18 \times 6.7^{-1}) = 7$. It is clear that the two different approaches can produce some ambiguities in the assessment of risks resulting from different types of radiation.

Analysis of Tumor Incidence Data

The easiest approach in the analysis of tumor induction studies in animals is to score the fraction of animals that develop a tumor or the mean number of tumors per animal observed after a given dose. However, this use of so-called uncensored data may lead to erroneous interpretation of results. During the observation time, the group of animals at risk may be reduced due to intercurrent deaths or losses of animals from the experiment due to reasons not related to the endpoint under investigation. Consequently, one deals with incomplete right censored data. Appropriate corrections for competing risks in the tumor rate analysis can be made by using life-table methods, such as the product limit estimate of Kaplan and Meier (1958) or the sum limit estimate. The resulting quantities, such as the cumulative tumor rate (Shellabarger et al. 1980) or the cumulative prevalence and actuarial incidence (Broerse et al. 1985), provide meaningful expressions of the response. The probability curves produced by the life-table analysis inevitably show discontinuities in the course of time. The stochastic effect of carcinogenesis, however, can be described by hazard functions which result in curves for several groups with a common shape. This can be done using nonparametric models, for example, the proportional hazards model (Kalbfleisch and Prentice 1980; Kelllerer and Chmelevsky 1982) or analytical models, for example, the Weibull distribution (Broerse et al. 1985). In the more recent analyses, the time elapsing from when an animal enters the experiment until a specified type of tumor becomes detectable is taken as the endpoint. One knows either the actual time that a tumor is observed in an animal or that the animal has not developed such a tumor up to its time of disappearance from the experiment. Methods of analysis that accommodate censoring are generally called failure time distributions. The applicability of various mathematical models for analysis of animal carcinogenesis data is discussed elsewhere (Chmelevsky and Morin 1991; Davelaar et al. 1991).

It becomes more difficult to determine the carcinogenic action of ionizing radiation when only a few tumors have been observed at high doses. This has been the case for the induction of mammary carcinomas in Sprague-Dawley rats (Broerse et al. 1982). Under these circumstances the tumor incidence data can be analyzed by employing contingency tables with a computer program developed by Peto et al. (1980). The number of animals dying with a specific tumor is compared with the number that would have been expected to die, taking into account the group size and the intercurrent mortality which does not have tumors of the type of interest as the underlying cause. On the basis of this approach, the carcinogenic effect of a specific agent would be clearly demonstrated only when the ratio of observed and expected events is in excess of 1 (Broerse et al. 1982).

In order to obtain complete information on *in vivo* tumorigenesis results, it will be essential to keep the animals under observation during their full life span and to include adequate control groups of comparable age distribution. Tumor incidence data should always be based on histopathological examinations. In most analyses, the moment of observation of a palpable tumor is taken as an endpoint. This implies that for microscopic tumors found upon necropsy a slightly different mathematical approach should be followed than the approach for grossly visible tumors (Broerse et al. 1986).

Nature of Dose-Response Relationships

When all appropriate corrections have been applied, the dose-response relationships for radiation-induced cancer can, in a general way (see also equation 7, Chapter II), be described as: $I(D) = (a_1D + a_2D^2) \times S(D)$, where $I(D)$ is the incidence of induced tumors after an absorbed dose D , a_1 and a_2 are coefficients for the linear and dose-squared terms of cancer initiation, and $S(D)$ is the probability of survival of cells at risk (UNSCEAR 1986). Application of this equation to experimental data will usually also require an allowance for spontaneous tumors and the possibility that the progenitor cells may already be present at the time of irradiation. It should further be recognized that hormonal, immunological, and dietary factors will have a major modifying influence on tumor incidence. The quotient of the induction constants (a_1/a_2) varies with the radiation quality and the specific biological effect. For tumor induction in most tissues, the influence of the cell kill factor will not be very important in the range of doses up to about 1 Gy of low-LET radiation (or high-LET equivalent doses). It has been pointed out (Upton 1977) that the linear-quadratic model of cancer initiation is a simplistic concept and not a theory of pathogenesis. The great complexity and the interaction of various phenomena at the cellular and tissue level preclude its applicability for cancer induction in all tissues and under all circumstances. An extensive review of dose-response relationships in experimental systems has been made by UNSCEAR (1986). The induction of skin cancer in rats and mice by local low-LET irradiation requires high doses, and apparent or real thresholds cannot be excluded, nor can they be established from existing data. For the induction of bone sarcoma after incorporation of ^{226}Ra and ^{90}Sr in dogs and mice, an apparent threshold is indicated.

In a restricted number of studies, tumor induction has been studied in the same tissue with radiations of different quality, notably x or gamma rays (low-LET) and neutrons or alpha particles (high-LET). The nature of the initial parts of the dose-response curves for carcinogenesis in various tissues with respect to the presence of a linear and/or quadratic coefficient are summarized in Table VIII-2.

In the intermediate and low-dose region, life shortening is essentially due to tumor mortality. This does not imply that dose-response curves for both endpoints are directly comparable because, even for the same type of radiation, the mean latent periods of some tumors vary with the age at the time of exposure and physical factors, such as dose or dose rate. A separate analysis of ovarian tumor induction from a life-shortening experiment in mice (Covelli et al. 1988) supported the presence of a threshold dose for x rays. The results of two other experiments on life shortening in mice are included in Table VIII-2.

Table VIII-2. Characteristics of the initial part of dose-response curves for carcinogenesis in various tissues after exposure to radiations of different quality.

	Low-LET		High-LET	
	Linear	Quadratic	Linear	Quadratic
Lung adenocarcinoma in mice				
Ullrich (1983)	+	+	+	—
Coggle (1988)	—	+	+	—
Lung carcinoma in rats				
Chmelevsky et al. (1984)			+	—
Myeloid leukemia in mice				
Mole (1984)	—	+	+	—
Ullrich and Preston (1987)	+	—	+	—
Mammary carcinoma in rats				
Broerse et al. (1985)			+	—
van Bekkum et al. (1986)	—	+		
Life shortening in mice				
Maisin et al. (1988)	—	+	+	+
Thomson et al. (1985)	+	—	+	—

+ Analysis of data supports presence of this coefficient.

— Analysis of data does not support presence of this coefficient.

For four experimental endpoints, notably mammary carcinoma in rats (van Bekkum et al. 1986), lung adenocarcinoma in mice (Coggle 1988), myeloid leukemia in mice (Mole 1984),

and life shortening in mice (Maisin et al. 1988), approximately pure quadratic dose-response relationships have been reported for low-LET radiation. The apparent absence of a linear term in the dose-response equation for tumor induction after x-irradiation is difficult to explain in light of the microdosimetric data. Apparently, in some cases, repair processes at the (sub)cellular level and systemic factors can modify the primary process of cancer initiation. Another important factor could be the killing of progenitor cells that otherwise give rise to the spontaneous frequency (Elkind 1987). The net effect of their removal and the relatively small increase of transformed cells could cause the low-dose region of the curve to appear flat.

In respect to the dose-response curves for induction of myeloid leukemia, it should be recognized that Ullrich and Preston (1987) irradiated their mice over a dose range up to 1 Gy of gamma rays, whereas Mole (1984) applied a more extended dose range up to 6 Gy of x rays. Mole observed concavity of the curve at low doses, a maximum in the incidence at doses between 2 and 3 Gy, and convexity at higher doses.

Relative Biological Effectiveness of Fast Neutrons

Contrary to directly ionizing radiation, such as alpha particles, approximately exponential depth-dose distributions are observed for neutrons which, as a result of their interactions in tissue, deposit their energy through recoil protons, alpha particles, and heavy ions. Therefore, fast neutrons can be considered a convenient radiation modality to irradiate a relatively large biological object with high-LET radiation.

RBE values can be estimated from the animal data at relatively low dose levels, for example, at a neutron dose of 10 mGy. RBE values derived with reference to gamma radiation will be higher than those assessed with regard to x rays because the RBE of gamma rays to x rays is smaller than unity. Furthermore, when the estimates are based on different assumptions, the same set of experimental data can result in widely differing RBE values. In reporting his data on lung adenocarcinoma, Ullrich (1983) derived an RBE of 18.5 on the basis of the coefficients of the linear terms of the neutron and gamma ray dose-response curves, whereas Fry (1981) quoted an RBE of 60 based on the same criteria. Assuming an inverse-square-root dose-dependence, Ullrich computed an RBE of 71 for a neutron dose of 10 mGy. The calculation of different RBE values from the same experimental studies implies that caution should be exercised in the interpretation of such data.

The studies performed by Shellabarger et al. (1980) on mammary neoplasms in Sprague Dawley rats have indicated a proportionality of RBE to the inverse-square-root of the neutron dose. Only a minor fraction of all mammary tumors observed in this study consisted of adenocarcinomas. However, the authors claimed that the RBE-dose dependence for fibroadenoma alone and the combined group of fibroadenoma and carcinoma is also consistent with the more limited data pertaining to adenocarcinoma. In a subsequent experiment, Shellabarger et al. (1982) investigated the effect of radiation and the administration of diethylstilbestrol (DES) in another rat strain. For ACI rats treated with DES, the RBE values were larger than those for Sprague Dawley rats at comparable neutron doses.

The highest RBE values are observed for neutrons with energies of 0.43 to 1 MeV, as produced by the p+T reaction or by the fission process. A listing of RBE values for neutrons with energies between 0.43 and 1 MeV, in most cases with reference to x rays, is given in Table VIII-3. Additional information on the comparative tumorigenic effects of fission neutrons and ^{60}Co gamma rays in mice was reported by Grahn et al. (1992). RBE values, derived from the ratios of linear coefficients of the dose-response curves, varied between 2.5 and 16 for lymphoreticular tissue tumors, between 4 and 14 for vascular tissue tumors, between 6 and 30 for epithelial tissue tumors, between 5 and 24 for lung tumors, between 5 and 26 for liver tumors, between 8 and 79 for tumors of the glandular and reproductive systems (except ovaries), between 2 and 10 for ovarian tumors, and between 7 and 100 for tumors of the Harderian gland for time intervals in excess of 800 days. RBE values increased as the dose was protracted, largely due to the reduced effectiveness of protracted gamma irradiation. However, about 28% of the increase can be attributed to the increase in neutron-induced injury caused by dose protraction. For the induction of myeloid leukemia in mice by x rays, Mole (1984) was able to fit the data for the induction process using only a quadratic term, that is, without a need for a linear term. The RBE values for induction of mammary tumors (Broerse et al. 1985) were based upon linear dose-response curves for neutrons and x rays. Appreciably higher RBE values would be obtained if the results of the gamma exposure indicating a quadratic dose-response relationship (Broerse et al. 1991) were used as a base line. It should be recognized, however, that such an increase in RBE would result from a lower efficiency of the low-LET radiation, rather than from an increase in the efficiency of the neutron exposures in producing mammary tumors at low doses.

Effects of Fractionation or Protraction of the Dose

When the radiation dose is administered in a number of fractions or at a reduced dose rate, the biological response is different from that obtained after single, acute doses. For cell survival, the modifying processes include repair of sublethal damage, repopulation, and redistribution over the cell cycle. For tumor induction, the nature of modifying mechanisms is not yet well understood, and it is rather difficult to interpret all experimental results obtained after fractionated or protracted exposures.

In general, the carcinogenic effect for low-LET radiation is reduced when a fractionated exposure, rather than an acute exposure, is applied. However, for more densely ionizing radiations, a number of experiments have been reported where a greater biological effect was observed after a protracted exposure than after the same total dose was delivered in an acute exposure. This inverse dose rate effect was first demonstrated by Hill et al. (1982) with the induction of *in vitro* oncogenic transformation by fission spectrum neutrons. However, data from other *in vitro* transformation studies using neutrons and alpha particles have not demonstrated an enhanced efficacy of low dose rate irradiation with high-LET radiation. The inverse dose rate effect and possible cellular processes underlying this effect are discussed in Chapter VI.

Table VIII-3. Relative biological effectiveness at a dose (D_N) of 10 mGy of neutrons with energies between 0.43 and 1 MeV for different endpoints.

	Reference Radiation	RBE at 10 mGy
Lung adenocarcinoma in mice, a_1 neutrons/ a_1 photons, $D_N^{-1/2}$ Ullrich (1983)	gamma rays	18.5 71
Lung carcinoma in Sprague Dawley rats Lafuma et al. (1989)	gamma rays	50
Malignant transformation in hamster embryo cells Borek and Hall (1984)	x rays	10-25
Myeloid leukemia in mice, daily chronic irradiation Upton et al. (1970)	gamma rays	16
Myeloid leukemia in mice, acute irradiation Mole (1984)	x rays	14
Myeloid leukemia in mice Ullrich and Preston (1987)	gamma rays	3
Mammary fibroadenoma in Sprague Dawley rats, $D_N^{-1/2}$ Shellabarger et al. (1980)	x rays	50
Mammary adenocarcinoma in ACI rats treated with DES, $D_N^{-1/2}$ Shellabarger et al. (1982)	x rays	100
Mammary adenocarcinoma in WAG/Rij rats Broerse et al. (1985)	x rays	15
Mammary fibroadenoma in WAG/Rij rats Broerse et al. (1985)	x rays	13
Mammary fibroadenoma in Sprague Dawley rats Broerse et al. (1985)	x rays	7
Life shortening in mice Thomson et al. (1985)	gamma rays	15
Life shortening in mice Covelli et al. (1988)	x rays	12

For high-LET radiation, the effects of fractionation and protraction (see Table VIII-4) are different for the various tumor types, presumably because of the different mechanisms of tumor induction that are involved. For the induction of ovarian tumors in mice, Ullrich (1984) observed that fission neutron irradiation was less effective when delivered at low dose rates in comparison with high dose rates. However, the mammary carcinogenic effect of neutrons was enhanced at low dose rates, a finding similar to that of Vogel and Dickson (1982). Studies on the induction of mammary carcinoma in WAG/Rij rats after single and fractionated irradiations with x rays and 0.5 MeV neutrons indicate that for equal total absorbed doses the tumors appeared at approximately the same age (Broerse et al. 1985).

Table VIII-4. The effect of fractionation or reduction in dose rate on tumor induction or longevity in experimental animals after high-LET radiation.

	Change in Effectiveness ^a
Mammary tumors	
Ullrich (1984)	+
Vogel and Dickson (1982)	+
Broerse et al. (1985)	=
Ovarian tumors	
Ullrich (1984)	-
Pulmonary tumors	
Ullrich (1984)	=
Little et al. (1985)	=
Lundgren et al. (1987)	+
Myeloid leukemia in mice	=
Lymphosarcoma in mice	-
Huiskamp (1991)	
Osteosarcomas	
Müller et al. (1990)	+
Life shortening in mice	
Thomson et al. (1985)	+
Maisin et al. (1988)	=

^a Enhanced (+), reduced (-) or equal (=) with respect to single or high dose rate irradiation.

It should be noted that the experimental studies on mammary carcinogenesis are generally based on whole-body irradiations of the animals. The induction of mammary cancer can easily be modified by hormonal factors, and it might well be that specific endocrinological effects caused by the irradiation influence mammary tumor induction to a lesser extent when fractionated or protracted exposures are studied.

The induction of lung cancer after single or protracted exposures to alpha particles was investigated independently in three species of rodents. Lundgren et al. (1987) studied the effect of inhalation of ^{239}Pu oxide in mice after single or repeated exposures. For similar cumulative doses from alpha particles, the incidence of pulmonary tumors was approximately four times greater for repeated exposures than for a single inhalation exposure. The effect of dose rate on the induction of lung cancer in Syrian hamsters was examined by intratracheal instillation of ^{210}Po (Little et al. 1985). In comparison to a 10-day exposure period, protraction of the alpha irradiation over 120 days was slightly more carcinogenic at lower total lung doses, and slightly less carcinogenic at higher total lung doses. The carcinogenic effect of a single intratracheal instillation of ^{210}Po was markedly enhanced by subsequent weekly instillation of saline alone, emphasizing the importance of noncarcinogenic secondary factors in the expression of radiation-induced lung cancer. Cross (1992) analyzed the incidence of lung tumors in rats after radon exposure and concluded that fractionation of the exposure is more efficient in producing tumors, but further fractionation leads to a decreased efficiency of tumor production. Cross stated that the implications of these findings for human risk assessment are somewhat uncertain at this time.

Extrapolation to Low Dose Levels

Risk estimates for radiogenic cancer at the low radiation exposure levels of main concern in radiation protection practice have depended on extrapolation from human data obtained largely at high doses and high dose rates. This procedure involves extrapolation over a factor of up to 1,000 in dose and of the order of a billion in dose rate.

An observed dose-incidence relationship based on fragmentary data may be compatible with various mathematical functions. Most of the human data are fragmentary, as are the data from many individual animal experiments. However, a review of the accumulated data from animal experiments, with emphasis on the more thorough and comprehensive experiments which involve thorough ascertainment of lifetime incidence and temporal advancement of neoplasms over wide ranges of brief, intensive exposures, suggests dose-effect relationships for low-LET radiations that are usually compatible with a linear-quadratic curve.

A large body of experimental evidence supports the inference that the effectiveness per unit dose of low-LET radiation for life shortening and cancer induction is reduced as the exposure rate is reduced. The reductions observed in different experiments have varied by up to a factor of about 20, depending on the effect, the dose range, and the differences in dose rates compared. Most of the experiments showed reductions of effect by factors in the range of 2 to 10 when the dose was in the range of 1 to 3 Gy.

With increasing reduction of dose rate the rising portion of the low-LET curve becomes more shallow (slope decreasing) and eventually flattens to a straight line which is often assumed

to be asymptotic to the linear, low-dose part of the single exposure, high dose rate induction curve (see Fig. I-1). When this assumption is valid, it is easier to determine the slope of that low linear part from data at high doses and low dose rates than at low doses and high dose rates. Owing to differences in mechanisms of oncogenesis in various tissues, the coefficient a_1 (see equations 5 and 7 in Chapter II) for low-LET radiation may vary considerably, and in rare cases may possibly be zero.

It has been assumed that the difference in effect between radiation qualities is quantitative and not qualitative. However, the validity of this assumption should always be kept in mind during investigations of RBE and mechanisms. That qualitative differences may exist is illustrated by at least two cellular systems, notably human lymphocytes and mouse hemopoietic stem cells. Sister chromatid exchanges could be induced by irradiation of unstimulated human lymphocytes with high-LET radiation, but apparently not at all with low-LET radiation (Savage and Holloway 1988; Aghamohammadi et al. 1989). Irradiation of hemopoietic stem cells of CBA mice by alpha particles can induce chromosomal instability that is expressed at high frequency as new aberrations many cell cycles later (Kadhim et al. 1992). Such lesions in stem cells resulting in the transmission of chromosomal instability to their progeny were not observed after x irradiation.

It has been found that RBE values for a number of effects, including cancer induction, are consistent with an inverse relationship to dose over the experimental range. However, the relationship depends on the shapes of the dose-effect curves for the test and reference radiations. For small doses, a maximum and constant RBE value would be reached if both dose-effect curves initially are linear.

The initial slopes of neutron dose-effect curves vary among tumor types by a factor of about 6, which is less than the variation with gamma radiation. In consequence, the RBE of high-LET radiation generally increases with decreasing dose and/or dose rate, from values in the range of 1 to 5 at high doses and dose rates to values in the range of 10 to 100 or more at the lowest doses and dose rates investigated so far. Such wide ranges of values highlight the problem of basing Q values on RBE values. The initial slopes of the tumorigenic responses for both high- and low-LET radiations have been determined only in a few studies and only with broad confidence limits.

The RBE of a particular radiation for tumorigenesis is dependent on LET, dose, tissue, dose rate and fractionation, and age. The individual tumor incidence per unit dose of radiation varies among tissues, and at low doses it varies more with low-LET radiation than with neutrons. Consequently, the RBE varies over a wide range. The precise relationships of RBE to LET have not been delineated.

No definitive model has been developed to take into account all of the factors which influence the shapes of the dose-effect curves for tumor induction, including the physical factors, such as the distribution of dose in the tissues and cells, and all of the biological factors, such as cell killing. The dose-effect curves reflect the net sum of all the factors that influence tumorigenesis, from initial events of induction to expression. The incidence of tumors is a poor measure of initiated cells that are capable of forming tumors or will cause tumors, and *vice versa*, because of other endogenous and exogenous factors that determine whether or not the induced transformations in initiated cells progress to the expression of neoplasms.

The development of dose-response models for cancer induction by high-LET radiations is hindered by a lack of suitable experimental data. Inadequacies in existing data stem from several sources. First, the commonly used absorbed dose model is inadequate for describing the effects of very low doses of high-LET radiation where a microdosimetric model would be more appropriate. Second, there are insufficient cancer induction data in various tissues, animal strains, and species at very low neutron doses (less than 0.1 Gy). Existing data are often incomplete due to premature termination of data collection. Where possible, studies should run for the natural lifetime of the test subjects to ensure that all the effects are recorded and the lifetime tumor induction may be determined. Studies which are prematurely terminated provide tumor incidence rates, but fail to provide much-needed data on tumor advancement. Also, premature termination tends to bias results by underestimating tumor-incidence rates in the control group.

GENETIC AND HEREDITARY EFFECTS

Introduction

UNSCEAR has produced a series of comprehensive reviews of data on the biological effects of ionizing radiations (UNSCEAR 1972, 1977, 1982, 1986, 1988), including data from genetic research on a wide variety of test systems. Selective reviews and analyses of these data have been done to establish recommendations for radiation protection (e.g., NAS/NRC 1972, 1980; ICRP 1972, 1977a, b). Still other reviews for that purpose have focused upon radiation quality and relative biological effectiveness (RBE) (e.g., Myers and Johnson 1986; ICRP/ICRU 1963; NCRP 1987a, 1990).

The information available on the genetic effects of radiation has led to numerous generalizations. Some of these are discussed below, although it is recognized that there may be apparent or actual exceptions.

None of the observed genetic changes is qualitatively unique or pathognomonic for radiation exposure, and, therefore, the determination of phenotypic changes due to radiation requires that these genetic effects be quantitatively established. However, ionizing radiation may produce relatively unique changes at the level of the base sequence of genes (see Chapter VI).

It has been possible to study the genetic effects of radiation in simple test systems and lower organisms with much greater precision than to study any of the other biological effects or genetic effects in higher, more complex organisms.

Dose-Response Relationships

The shape of the dose-effect relationship curve for low-LET radiation at higher doses and high dose rates varies considerably for different genetic changes scored in different biological systems, and so does the RBE for neutrons. As an example, the induction of gene conversion in yeast appears to be strictly proportional to radiation dose over a wide range of sublethal

doses, to be relatively independent of dose rate, and to have a low RBE for neutrons (Myers and Johnson 1986). On the other hand, the induction of stamen hair mutations in *Tradescantia* has both linear and dose-squared components in the dose-effect relationship for low-LET radiation at high dose rates, and the dose-squared component disappears at low dose rates. The RBE for fast neutrons, in relation to x rays at low doses or low dose rates, approaches 50 in the *Tradescantia* system, but is less than two in the yeast conversion system. The RBE of 1.3 for induction of back mutations in yeast by 14 MeV neutrons is also low. It has been noted (Myers and Johnson 1986) that gamma radiation at low dose rate is less effective than x radiation for the induction of mutations in *Tradescantia*, visible translocations in chromosomes of mouse spermatogonia, and visible chromosomal abnormalities in human leukocytes, but not for the yeast system.

It appears that the genetic effect of single doses of neutrons in mammals saturates at about 0.5 to 2 Gy, although the response to multi-fraction or protracted exposures appears to lie along a continuation of the linear portion of the curve to higher total doses. With this exception, there appears to be no general dose rate or fractionation effect for neutron irradiation (Myers and Johnson 1986). Neutron effectiveness per unit dose appears to be independent of dose size and dose rate in the dose-effect range below the saturation level and, therefore, may be a constant for any given genetic test system. Neutron RBE values increase as the dose and/or dose rate decrease to low levels, owing to the decreasing effectiveness of the low-LET reference radiation. Neutron RBE values appear to be invariant at low doses and are equal to the ratios of the slopes of the linear portions of the dose-effect curves for the neutron and reference low-LET radiations.

For technical reasons, data on the induction of forward mutations in cultured mammalian cells are limited. The shape of the dose-effect curve for low-LET radiation at high dose rate and the RBE for neutrons differ considerably, depending upon the type of mutation, even in the same cells. For example, mutations in the gene for dihydrofolate reductase tend to increase in direct proportion to the dose of gamma radiation and to have a low RBE for neutrons (Myers and Johnson 1986). In contrast, the response for mutations in the gene for hypoxanthine-guanine phosphoribosyl transferase after gamma radiation exposure is similar to that described for somatic mutations in *Tradescantia* and shows a moderately high RBE for neutrons (Cox et al. 1977).

Chromosomal Aberrations

Radiation induction of stable chromosomal aberrations involving the translocation of genetic material is of interest because translocations contribute a considerable part of the total genetic hazard from radiation exposure (UNSCEAR 1982), translocation of oncogenes is thought to be a mechanism in cancer induction (UNSCEAR 1986), and induction of chromosomal abnormalities can be studied both *in vitro* and in intact animals with greater precision and at lower radiation doses than can the induction of genetic changes in the offspring of irradiated animals.

Genetically transmissible, balanced reciprocal chromosome translocations induced in spermatogonia can be scored readily at the first meiotic metaphase. Since the report of Evans et al. (1964), there has been an extraordinary increase in the amount of data on this genetic endpoint obtained in mice and other mammals. Most of the data have been summarized in considerable detail in UNSCEAR reports (UNSCEAR 1972, 1977) and more briefly, for low-LET radiation, in NCRP documents (NCRP 1980, 1990) with focus on the effect of neutrons and alpha particles in comparison with the effects of single, weekly, and continuous gamma irradiation.

In mice, for high doses delivered at high dose rates, x rays and gamma rays are almost equally effective for the production of these translocations (UNSCEAR 1972). With a decrease in dose rate, the effectiveness of x rays decreases by a factor of about 2, while that of gamma rays decreases by a factor of about 10, thereby making gamma rays roughly one-fifth as effective as x rays at low dose rates (UNSCEAR 1972, 1982). The RBE of 0.7-2 MeV neutrons for induction of translocations in mouse spermatogonia has been found to be in the range of 3-10 relative to x rays at low dose rates, or in the range of 20-80 relative to gamma rays at low dose rates (UNSCEAR 1972, 1977). Grahn et al. (1983), using weekly dose fractions of fission neutrons and ^{60}Co gamma rays over a period of 60 weeks, found neutron RBE values of 10-24 for induction of translocations in mouse spermatogonia.

The general appearance of dose-response curves for reciprocal chromosome translocations induced in spermatogonia has been essentially repeated or confirmed in all studies (NCRP 1990). Single radiation exposures induce a generally linear increase with doses up to about 6 Gy for low-LET radiations and about 1 Gy for high-LET radiations. Above these dose levels, the response drops rapidly. This maximum in the curve at the higher dose levels has been observed for a variety of genetic and stochastic somatic endpoints and is attributed to the killing of cells. Protraction and/or lengthy fractionation of the dose usually causes continuation of the linearity of the dose-effect curve to much higher dose levels.

The linear dose-effect response observed at the lower single-dose levels of low-LET radiation may be a result of the intrinsic heterogeneity of radiosensitivity of the spermatogonia. If the cells are scored at or near the first major wave of meiosis after radiation exposure, a classical linear-quadratic response prevails up to approximately 5 Gy of x rays (Preston and Brewen 1973) and 3 Gy of gamma rays (Grahn et al. 1984). Because most studies have used a nearly constant interval between irradiation and sampling, regardless of the dose, the cell population has been more heterogeneous, as a result of having passed through a number of mitotic divisions before reaching the first meiotic metaphase. This procedure could have obscured the fine distinction needed to detect the nonlinear component of a dose-response curve.

Information on chromosomal aberrations is also available for the female mouse. Dose-response curves for x ray, gamma ray, and neutron-induced chromosome aberrations in maturing, as well as immature, mouse oocytes have been presented (e.g., Brewen et al. 1976; Brewen et al. 1977; Straume et al. 1991). The dose-response relationships for neutrons, acute x rays, and chronic gamma rays, are as expected radiobiologically, that is, neutrons are most effective, acute x rays are intermediate, and chronic gamma rays are least effective. The limiting RBE (i.e., RBE_M) for neutron-induced aberrations in immature mouse oocytes equals 16 for 0.43 MeV monoenergetic neutrons (Straume et al. 1991).

Reducing the dose rate and the size of dose fractions predictably acts to reduce the effect per unit dose for low-LET radiations because of the multi-track component of injury. This is especially notable for continuous, low-intensity gamma irradiation (NCRP 1990). The dose rate effectiveness factor for gamma radiation delivered at approximately 1 Gy per minute, compared to approximately 0.01 mGy per minute, is between 10 and 15 (Brewen et al. 1979). In direct contrast, protraction of neutron irradiation augments the damage, especially at high doses. RBE values for single doses of neutrons, compared to ^{60}Co gamma rays, are usually between 3 and 6. The value would approach 8 to 10 at extremely low doses (NCRP 1990).

Although genetic changes cannot be studied in higher organisms, such as mammals, with the same degree of precision as in simple systems, the dose-effect relationships could be fitted to the same general types of curves (Myers and Johnson 1986). The results of studies of the genetic effects of radiation exposure in animals have been reviewed frequently because of the critical importance of the data, especially those on mice, in the assessment of genetic risks from radiation exposure to people.

Dominant Lethal Mutations

Since the report by Russell et al. (1954), studies by various investigators have repeated the general finding of an RBE of 4 to 6 for single doses of fission neutrons compared to x rays for induction of dominant lethal mutations, including meiotic and postmeiotic male germ-cell stages.

Grahn (1983) summarized comparisons of single and weekly exposures to fission neutrons with a mean energy of 0.85 MeV with single, weekly, and continuous exposure to ^{60}Co gamma radiation. Weekly and continuous exposures were carried out for 30 to 50 weeks, and dominant lethal tests were performed at intervals during that period. For postspematogonial stages, the RBE for neutrons was 5 for single exposures, 10 for weekly exposures, and 15 when comparing weekly neutron exposure with continuous low-dose-rate gamma irradiation. Since no continuous neutron exposure data are available for direct comparison, the RBE value of 15 is based on the assumption of additivity for periodic or fractionated neutron exposures. That assumption may be regarded as reasonable because the mutation rate following weekly neutron exposures does not decline as it does following weekly gamma radiation exposures, in comparison with their single-dose values. In fact, the mutation rate for the weekly neutron exposure may be as much as 1.5 fold greater than that for the single dose, which accounts for part of the increase in the RBE from 5 to 15.

The assumption of neutron dose additivity may hold for comparisons involving spermatogonial irradiation, and the RBE values are more dependent upon the pattern of exposure to the low-LET radiation (Grahn 1983). RBE values estimated from the ratios of dose-response regression coefficients, as they were for those noted above for mature cell stages, are about 4 for single exposures, 15 for weekly exposures, and 30 for the comparison of weekly neutron exposures to continuous gamma irradiation. An important feature of these data on induction of dominant lethal mutations in stem cells is that they conform to linear dose dependencies so that RBEs are the same over a wide range of doses.

A study by Grahn et al. (1984) on the effects of extremely low single doses of neutrons compared to a limited array of ^{60}Co gamma ray doses has confirmed the previous RBE value of 5 as prevailing over the full range of neutron exposures for dominant lethal mutations induced in the postspematogonial germ cells of mice. There is a possible exception to this that may be a significant consideration for occupational exposure standards. The responses at 10 mGy and at 25 mGy deviate from linearity with a mutation rate in excess of expectation. This deviation is statistically significant, but does not exceed a factor of two above the expected. The dose-effect curve has a small "blister" above the linear trend at these two lowest dose levels. At the lowest neutron doses, the RBE is 10, while it is 5 at all dose levels above 50 mGy. The data at 10 and 25 mGy conform to the dose-response regression describing the response to low doses of neutrons delivered at 1 to 50 mGy per week (Grahn 1983). Therefore, this phenomenon of a response in excess of expectation may be a more general one at low doses and may influence RBE values significantly.

Data on neutron-induced dominant lethal mutations in oocytes are sparse. Searle and Beechey (1974) reported on the induction of dominant lethals in maturing mouse oocytes by single doses of fission neutrons (mean energy 0.7 MeV). Although the data were not analyzed statistically, the authors noted that the RBE would be about 1 compared to x rays for the induction of all lethal mutations (pre- and postimplantation) among litters conceived during the first week after radiation exposure. However, a full regression analysis of only the postimplantation fetal losses yields an RBE of 4.8, derived from the ratio of slopes of the dose-effect curves. The nonirradiated control group for the neutron exposure groups showed an unusually high level of fetal mortality. Use of the nonirradiated control group for the x ray series for both the neutron and the x ray analyses yields an RBE of 5.6. Straume et al. (1991) reported that dominant lethals were induced in mouse immature oocytes irradiated by 0.43 MeV neutrons. As observed by Searle and Beechey (1974) for maturing oocytes, most (two-thirds) of the losses were due to preimplantation deaths. Straume et al. (1991) did not provide RBE values for dominant lethals, but they stated that the neutrons were substantially more effective than x rays when compared to a previous study (Goldstein 1987) using x rays and the same experimental methodology.

Thus, for single radiation doses, both sexes of mice appear to show similar RBEs for the induction of dominant lethal mutations. For protracted exposures, the RBE should increase by a factor of 5, which is the dose rate factor for the induction of dominant lethal mutations in the maturing oocyte after low-LET radiation (NCRP 1980). The dose reduction factor is closer to 7 if a regression analysis is used and an x ray versus gamma ray RBE of 1.5 is applied.

Specific Locus Mutations

The method for measuring the frequency of specific locus mutations has involved screening mice for mutations at any of seven specific loci in germinal cells, primarily spermatogonia and oocytes. The specific locus endpoint is thought to consist primarily of chromosome rearrangements, namely, deletions, duplications, insertions, etc., aberrations which include the specific locus under observation, but frequently extend to adjacent loci. A larger

proportion of the mutations induced in spermatogonia or oocytes consists of multi-locus deletions after neutron irradiation than after x irradiation (UNSCEAR 1977, 1982). The RBE values associated with specific locus endpoints for a given germ-cell stage mirror those found by induction of dominant lethal mutations and translocations.

Seven experiments have been reported in which specific locus mutations from the irradiation of spermatogonia were studied (Russell 1965; Batchelor et al. 1967). In Russell's experiments, doses of 0.52-0.55 Gy of fission neutrons produced essentially the same mutation rates when dose rates of 0.79, 0.008, or 0.002 Gy per minute were used. In the experiments of Batchelor et al. (1967), the mutation rate after a neutron dose of 0.62 Gy at a dose rate of 1×10^{-5} Gy per minute was consistent with the data from Russell's experiments. These data indicate no reduction in yield of specific locus mutations from neutron irradiation over an 80,000 fold dose-rate change, unlike low-LET radiation. Furthermore, the five experiments which included neutron doses ranging from 0.52-0.88 Gy yielded essentially the same rate of mutation per locus per Gy, indicating that over this narrow dose range there was no evidence of a departure from linearity of response. After a higher neutron dose (1.88 Gy) at a high dose rate (0.57 Gy per minute), the mutation rate was markedly decreased from that at lower doses. After a higher dose at a low dose rate (2.14 Gy at 2×10^{-5} Gy per minute), the mutation rate was significantly lower than would be predicted by linear extrapolation from the lower doses. Clearly this means that at either high or low dose rates, high doses may selectively eliminate mutated germ cells from the stem cell population, unlike the circumstances observed at lower total doses. Excluding the 1.88 Gy and 2.14 Gy doses, the RBE calculated for the fission neutrons, compared with chronic gamma irradiation, was between 17 and 23 (NCRP 1990).

Studies of specific locus mutations in mouse oocytes after either low-LET or high-LET radiations have shown a marked difference between oocytes recovered within the first 6 weeks after irradiation and those recovered at 7 or more weeks after irradiation. Those recovered within the first 6 weeks are mutable, while the later oocytes (early dictyate oocytes) show no mutational response to irradiation (NCRP 1990). It is likely that the immature mouse dictyate cells do not survive radiation damage to their cell membranes (Straume et al. 1991), and, therefore, their lack of mutational response does not describe either a genetic insensitivity or remarkable repair capacity. Although it has been stated that the response of the immature oocytes of the mouse cannot be extrapolated to the human situation (NCRP 1990), more recent studies indicate that the genetic information obtained for these cells could be extrapolated if damage to the plasma membrane is avoided through careful experimental design (Straume et al. 1991).

For x ray exposure, the mouse oocytes at 2-6 weeks postirradiation grossly show about a twofold greater sensitivity than those recovered in the first week. Data from neutron studies have not yet been reanalyzed this way and, therefore, cannot yet provide an estimate of RBE for different oocyte stages. Data obtained after acute neutron doses (0.3 to 1.20 Gy) show an increase in mutation rate with increasing dose, but it is not clear at 1.2 Gy whether or not the curve has begun to bend over, that is, to show evidence of a saturation of effect. A comparison of the neutron dose of 0.6 Gy at 1.5 mGy per minute with the same dose delivered at 0.79 Gy per minute shows a lower effect at the lower dose rate. The mouse oocyte data permit a wide

range of estimated RBE values. Using recent x ray data yields an RBE of 34, about midway between the other estimates (NCRP 1990).

RBE Values

The RBE of greatest interest in radiation protection is that for continuous or protracted exposure. The best estimates are those derived from the ratio of dose-response coefficients, where the individual regression is based upon 5 or more data points; a criterion that cannot be met consistently, however (NCRP 1990). A wide range of RBE values is obtained by mixing the results from studies that are separate with respect to investigators, time, places, radiation source, mouse strain, etc. For fission spectrum neutrons, one approach would be to take the mid-range value for long-term weekly or continuous exposure to the two radiations that are being compared. A nominal RBE of 50, with a range of 30 to 70, would accommodate the majority of the available data (NCRP 1990). This nominal value might be considered to be a maximum value, as the RBE would fall to lower limits of 10 to 30 when the comparison involves large periodic dose fractions of gamma rays, for example, at levels above 0.4 Gy per week (Grahm 1983).

In partial summary, there is the consistent finding of an RBE of about 5 (range 3 to 6) for fission neutrons, compared to ^{60}Co gamma rays, following single doses at high dose rates, for the endpoints of dominant lethal mutations induced in the meiotic and postmeiotic male germ cell stages and for reciprocal chromosome translocations and dominant lethal mutations induced in spermatogonia. At the other extreme, that is, the comparison of protracted (fractionated) neutron doses with continuous gamma irradiation at low dose rate, the RBE values are about 15 for dominant lethals induced in mature germ-cell stages, 30 for dominant lethals induced in spermatogonia, and up to 50 for reciprocal translocations.

Reduction of the dose rate and/or total dose of low-LET radiation clearly reduces the mutagenic effectiveness of the radiation, while the same response does not occur for the high-LET radiations. The RBE, that is, the ratio of the neutron/gamma dose-effect slopes, must therefore steadily increase until the minimum slope value for the low-LET radiation is reached.

An exception to this description was observed for weekly neutron exposures delivered over a long period of time where the mutagenic effectiveness apparently increased by a factor of 1.5 to 2 over that of single neutron doses at low dose levels. This is not a universal finding, but taking it into account could increase the RBE for certain comparisons against low-level continuous gamma irradiation. For example, in a comparison of the maximum dose-response slope for the induction of translocations by neutrons and the minimum for gamma rays at low dose rate, an RBE of about 75 can be derived (NCRP 1990).

RBE values of fission neutrons versus gamma rays for induction of mutations in spermatogonia are in the vicinity of 20 for neutron doses of 0.5-1.0 Gy, and over this dose range the mutation rate is independent of dose rate over a wide range.

As indicated above, the immature oocytes (the principal genetic pool in both female mice and in women) of the mouse are killed by radiation damage to an unusually sensitive cell membrane (Straume et al. 1987, 1989, 1991). Therefore, neutrons, such as those from most

fission sources that produce proton recoils with tracks longer than a cell diameter, are unlikely to result in a detectable genetic response (Straume et al. 1991). However, neutrons having energies below a few hundred keV produce subcellular recoil protons that can damage DNA without simultaneously killing the cell via plasma membrane traversal. This has now been demonstrated using 0.43 MeV neutrons for chromosome aberrations and dominant lethals in mouse immature oocytes (Straume et al. 1991) and paves the way for specific-locus testing of these cells.

For maturing mouse oocytes (which make up only a very small part of the genetic pool), neutron RBEs are obtainable from available data (e.g., see Searle and Beechey 1974; Lyon et al. 1979; NCRP 1980, 1990). Point-estimates of RBE_M for fission neutrons range from 14 to 70, possibly reflecting differences in age of the host and in sensitivity of cell stages. The rate of induced mutations per locus per unit dose for the maturing oocyte over the limited neutron dose range studied is similar to that observed for spermatogonia, that is, $(140 \text{ to } 200) \times 10^{-6} \text{ Gy}^{-1}$ for maturing oocytes and $160 \times 10^{-6} \text{ Gy}^{-1}$ for spermatogonia. Also, the RBEs observed for spermatogonia (17 to 23) are within the range of values obtained for oocytes. These findings, together with data from immature oocytes indicating that their intrinsic mutability and RBE relationships are similar to those for maturing oocytes (Straume et al. 1991), substantially strengthen the extrapolation of mutagenesis data from female mice to humans.

RADIOPROTECTION AGAINST LATE EFFECTS INJURY

The development of prophylactic and therapeutic regimes for persons who may be exposed to or irradiated with neutrons could considerably reduce late-effects injury. Chemical agents are well known modifiers of radiation injury. For example, dimethyl sulfoxide (DMSO), an efficient scavenger of hydroxyl radicals, is reported to protect rodent cells *in vitro* from radiation-induced mutations (Nakamura and Okada 1981; Sapora et al. 1991). Cell mutations *in vitro* can be reduced when irradiations are performed under anoxic conditions (Sapora et al. 1991). However, aminothiols treatment appears to be the most useful of the potential radioprotector therapies available.

The aminothiols, which are effective chemical antioxidants, have been demonstrated to be effective antimutagenic and anticarcinogenic agents (see review by Grdina and Sigdestad 1989). A summary of the findings for the radioprotective properties of aminothiols or their respective phosphorothioates following exposure to low LET-irradiation is shown in Table VIII-5. Depending on the late effects radiation injury endpoint and model system examined, protection factors range from 1.43 to 9.7.

Similar animal lifespan and *in vitro* cell mutation and transformation studies have been performed following exposure to fission neutrons (Table VIII-6). Figure VIII-3 compares low-LET versus fission neutron irradiation induction of mutations in the HGPRT locus in Chinese hamster V79 cells, where equal protection efficacy for WR-1065 was found (Grdina et al. 1988). With an *in vivo/in vitro* model system of splenic T-lymphocytes from B6CF mice, WR-2721 protection factors decreased from 2.4 to 1.4 following exposure to either ^{60}Co gamma rays or

JANUS neutrons, respectively, according to the HGPRT locus mutation assay (Kataoka et al. 1992).

Table VIII-5. Amino-thiol radioprotection from late effects injury: low LET-irradiation.

Endpoint	Reference Radiation	Compound	Protection Factor
Life shortening/mice Maisin et al. (1977)	x rays	Mixture ^a	1.5-2.5
Tumor induction/mice Maisin et al. (1978)	x rays	Mixture ^b	1.4
Tumor induction/mice Milas et al. (1984)	¹³⁷ Cs gamma rays	WR-2721	3.1
HGPRT mutation/V79 cells Grdina et al. (1985b)	⁶⁰ Co gamma rays	WR-1065	5.1
Preneoplastic lesions in rat livers Grdina et al. (1985a)	⁶⁰ Co gamma rays	WR-2721	9.7
HGPRT mutation/V79 cells and C ₃ H10T $\frac{1}{2}$ cell transformation at 4 Gy Hill et al. (1986)	⁶⁰ Co gamma rays	WR-1065	6.0
Tumor induction and life shortening/mice Grdina et al. (1991b)	⁶⁰ Co gamma rays	WR-2721	-
HGPRT mutation in mouse splenic T-lymphocytes Kataoka et al. (1992)	⁶⁰ Co gamma rays	WR-2721	2.4
Tumor induction/mice Hunter et al. (1992)	¹³⁷ Cs gamma rays	WR-2721	1.75

^a Mixture consists of glutathione, cysteine, 2- β -aminoethylisothiuronium-Br-HBr (AET), 5-hydroxytryptamine, mercaptoethylamine, and cysteamine.

^b Mixture consists of glutathione, cysteine, AET, cysteamine, and 5-hydroxytryptamine.

The anticarcinogenic properties of aminothiols following exposure to ionizing radiation have also been demonstrated. With the C₃H10T $\frac{1}{2}$ cell model system, WR-1065 protection factors decreased from 6 (Hill et al. 1986) to 3.2 (Balcer-Kubiczek et al. 1993) for ⁶⁰Co gamma rays and fission neutrons, respectively, for neoplastic cell transformation *in vitro*. These results are consistent with the conclusions by Grdina that WR-151327-induced enhancement of life span

Table VIII-6. Aminoethiol radioprotection from late effects injury: fission neutrons.

Endpoint	Reference Radiation	Compound	Protection Factor
HGPRT mutations/V79 cells Grdina et al. (1988)	ANL fission neutrons	WR-1065	3
HGPRT mutations/V79 cells Grdina et al. (1989)	ANL fission neutrons	WR-1065, WR-151316	2.7
C ₃ H10T ^{1/2} cell transformation Balcer-Kubiczek et al. (1993)	AFRRI fission neutrons	WR-1065	3.2
Carcinogenesis and life shortening/mice Grdina et al. (1991a)	ANL fission neutrons	WR-151327	-
HPRT mutation in mouse splenic T-lymphocytes Kataoka et al. (1992)	ANL fission neutrons	WR-2721	1.4
HPRT mutation in mouse splenic T-lymphocytes Grdina et al. (1992)	ANL fission neutrons	WR-2721	-

following irradiation by ANL fission-spectrum neutrons can be attributed to protection against neutron-induced tumor formation (Grdina et al. 1991a).

Figure VIII-3. Differential effects of mutagenesis by high-LET JANUS fission neutrons and by low-LET ⁶⁰Co gamma rays at HGPRT locus in V79 cells as a function of the presence or absence of WR-1065 (4mM) during irradiation. Cells were irradiated at 37°C and assayed for survival and mutagenesis using standard techniques (Grdina et al. 1988).

RESEARCH NEEDS

(1) In all future studies, it is essential that the accuracy and exposure conditions of neutron irradiations, including dosimetry and radiation quality, be verified. Adherence to dosimetry protocols or participation in dosimetry intercomparisons should be practiced. If required, a site visit should be made by a physics team to check the dosimetry, even in retrospect.

(2) The results of *in vivo* studies of carcinogenesis should be analyzed by actuarial methods and different mathematical models, for example, the proportional hazards model or analytical models, such as Weibull distribution.

(3) A limited number of experiments on *in vivo* carcinogenesis should be initiated that apply various dose rates and/or multiple fractions of low- and high-LET radiation, and compare species, for example, mouse and rat. Useful information could also evolve from the wealth of tissue samples that were collected during previous experiments, but were not analyzed because they were peripheral to the original study or because of a lack of time or funds.

(4) Studies of RBE_M should be initiated for dominant mutations and changes in activity of enzymes in mouse spermatogonia at relatively low dose levels.

(5) Long-term epidemiology on radiation workers and cancer patients treated with external beams of fast neutrons could provide limited, but valuable, data on human carcinogenic risk.

(6) Specimens and data from persons exposed to neutrons either accidentally or radiotherapeutically should be utilized for the purpose of studying chromosomal defects in peripheral lymphocytes and germ cells, if available.

(7) The nature of radiation-induced dominant and recessive mutations at defined gene loci should be investigated employing modern molecular techniques, as discussed in Chapters V and VI.

(8) Human data emerging from the early Soviet nuclear weapons programs at Chelyabinsk should be evaluated.

(9) Aminoethiol radioprotection studies using molecular, cellular, and animal model systems should be directed at providing an effective means to reduce risks from late effect injury following radiation exposure, based on the understanding of the mechanism of protection from cell mutation and neoplastic transformation.

Chapter IX

SUMMARY OF NEEDED RESEARCH*

This chapter summarizes the research needed in neutron radiobiology that was identified and described in more detail in the prior field-specific chapters. Along with each research need, this chapter also includes relative comments that either identify the need for the research or the specific scientific question the research will help elucidate. The research needs described in the present chapter were considered comprehensively in the process of determining the recommended integrated approach which is given in Chapter X. It will be useful for researchers in neutron radiobiology to consider the research needs listed in this chapter in the process of implementing the recommended integrated approach to examining the biological effectiveness of neutrons.

* The major part of the material in this report was prepared prior to June 1991.

CHAPTER II

RADIOBIOLOGICAL PRINCIPLES

Conventional methods of estimating human risk from neutrons and other radiations rely on a number of simplifying assumptions by which the limited available data on biological effects are extrapolated to practical situations of low doses and low dose rates. These simplifications rely on corresponding assumptions regarding the underlying radiobiological mechanisms. The validity and generality of these assumptions need to be tested for a variety of biological effects, particularly those most closely related to human tumorigenesis and mutagenesis.

Research Needs	Comments
1. Observed responses at the lowest experimentally-accessible doses of neutrons should be compared with expectations based on the linear term derived from fits at significantly higher doses for relevant effects. When deviations are observed, mechanistic explanations and their broader implications should be sought.	1-3. It is commonly assumed that the linear term of dose response, as derived from fits to data at intermediate and high doses and high dose rates, is constant down to zero dose and is independent of dose rate. This assumption needs to be tested for a wide variety of relevant biological systems.
2. Observed responses of neutrons at low dose rates should be compared with expectations based on assumption of a dose-rate-independent linear term as derived from fits at higher dose rates for relevant effects.	
3. Observed responses of low-LET reference radiations and mixed fields, at low doses and low dose rates, should be compared with expectations based on dose- and dose-rate-independent linear terms as derived from fits at higher doses and dose rates for relevant effects.	

4. Methods should be developed to observe the biological effects of single, or exact small numbers of, well-defined proton and alpha-particle tracks through individual cells for relevant effects.
 5. Methods should be developed to investigate the effect of time interval between individual tracks of well-defined protons and alpha particles through cells irradiated with exact small numbers of tracks.
 6. The ability of a multiple-track gamma-ray background to modify the effects of single protons and alpha particles should be investigated.
 7. Possible roles of multiple-track processes at intermediate or lower doses of neutrons, protons, alpha particles or reference radiations should be investigated. Processes to consider include induction or enhancement of repair, or of misrepair or damage fixation, dose thresholds, promotion of expression, and cell sterilization.
 8. Possible roles of background gamma-ray irradiation on the single- and multiple-track processes of neutrons, protons, and alpha particles should be investigated.
- 4-6. In situations of practical relevance, individual cells are irradiated almost exclusively by single tracks or small numbers of tracks spread over a long time. The responses of cells to single tracks, of the types which occur in neutron irradiations, need to be determined as directly as possible and compared to the responses to small numbers of tracks.
 - 7-8. If significant multiple-track processes occur below, or in the lower dose portions of available dose-response data, then simple linear interpolation of the fitted linear term to zero dose may be misleading. The possible presence and magnitude of such processes need to be investigated in relevant biological systems.

9. Qualitative differences should be sought in final biological consequences of high- and low-LET radiations, including even the possibility of some being unique to high-LET radiations.

10. The role in radiation tumorigenesis of damage to multiple cells should be investigated.

9. A characteristic feature of high-LET radiations is the high local densities of energy deposition by single tracks. Use of RBE concepts to compare radiations at the same level of effect assumes that the biological consequences of the high-LET radiations are identical to those produced by the sparsely distributed energy from very much larger numbers of low-LET tracks. The general validity of this assumption needs to be investigated for relevant biological effects.

10. It is commonly assumed that a radiation-induced tumor arises from radiation damage to only a single cell, and it is on this basis that single- and multiple-track mechanisms are evaluated. The validity and generality of this assumption need to be tested for neutron- and reference-radiation induced tumors of different types.

CHAPTER III

ORGANIZING DATA FOR PREDICTION OF RISKS

Qualitative and quantitative models of the response of biological systems are used both to help in organizing and evaluating experimental observations and to provide extrapolations to the effects of irradiation conditions (e.g., low doses or low dose rates) where direct experimental observations cannot be made. Suitably tested and verified models will be useful in determining the effectiveness of neutrons relative to other radiations and how this effectiveness varies under different irradiation conditions, for different species and for different health effects.

Research Needs	Comments
1. Phenomenological models of the relationship between tissue characteristics and probability of malignant transformation should be developed.	1-4. Satisfactory phenomenological models exist for many of the steps leading from radiation exposure to health effects, but other steps are only now being recognized and experimental techniques to investigate others have recently been developed. Phenomenological models should be developed for the remaining steps leading to health effects.
2. The effects of sublethal damage on cell-cell communication and regulation should be investigated and modeled.	
3. Factors affecting the expression of damage in different species should be identified and their relationships described quantitatively.	
4. Promotion, progression, and other effects which modify the effect of initial damage should be modeled.	

5. Models describing hypothesized mechanisms of oncogene activation and antioncogene inactivation should be developed.
 6. Mechanistic models of biochemical repair systems and their effect on cell survival, mutation, and transformation should be refined.
 7. The effects of cell-cell communication and other regulatory mechanisms on the expression of radiation damage should be studied.
 8. Growth factors and other modifiers of cell proliferation should be included in models of effects on tissues.
 9. The mechanisms underlying immune response and recognition in initiated cells should be modeled.
 10. Special attention (and funding) should be given to documentation at the end of major experiments.
 11. Funding should be provided for organizing and publishing workshops that feature applying results from other fields to understanding radiation effects.
 12. A national data base (similar to those existing in nuclear physics) should be established to make available the results in radiation biology and related molecular and cellular mechanisms.
- 5-9. A great deal is now being learned about the effects of specific types of biochemical damage and about the role of oncogenes and cytogenetics in the development of adverse health effects. Quantitative models of specific mechanisms can be developed and compared with emphasis on identifying characteristics which can be used to design experiments to establish the significance of the specific mechanism.
 - 10-12. Modeling relies on the availability of experimental data, sometimes seemingly irrelevant data, to guide the development of new models and to provide tests as the models develop.

CHAPTER IV PHYSICS AND DOSIMETRY

An understanding of the principles governing the interaction of radiation with biological systems, as well as accurate measurements of that interaction, is essential to the analysis of the effects of different types of radiations.

Research Needs	Comments
1. Atomic cross sections, attenuation coefficients, and kerma factors for high energy neutrons need to be measured.	1-2. Neutron interaction cross sections are needed both for accurate evaluation of doses and for input to models of energy deposition at the cellular and molecular levels. Most are available, but additional data, especially for neutron energies above 14 MeV, are needed.
2. Nonelastic and spallation cross sections resulting in high-LET secondaries should be determined.	
3. Ionization and excitation processes in condensed phase, particularly the collective effects in aqueous systems and macromolecules, should be measured.	3-6. The spatial distribution of ionizations and excitations produced by the charged secondaries of neutrons are responsible for their biological effectiveness. The cross sections for those interactions which may produce unique energy deposition patterns must be explored.
4. Cross sections for multiple ionization and other processes which lead to high local concentrations of chemical changes should be measured.	
5. Analysis of the sensitivity of energy deposition models and biological response models to the uncertainties in cross section data should be performed.	
6. The energy deposition processes and patterns which correlate most directly with biological effects should be determined.	

7. Compact detectors which can be used to map changes in the spectrum within the irradiated sample should be developed.
 8. Detectors which provide improved discrimination between photon and neutron events should be developed.
 9. Experimental confirmation of energy deposition distributions in all size sites down to a few nanometers should be made.
 10. Accurate evaluation of doses in relevant chemical compositions, such as in tissue rather than in conducting plastics, should be made.
 11. Improved area monitors which record LET spectrum or other characterization of the radiation should be made available.
 12. Improvements in size, weight, reliability, and cost of instruments are needed so that actual measurements can replace excessively conservative estimates.
 13. Personnel dosimeters which provide improved dose equivalent or, preferably, neutron spectrum data should be developed.
 14. Improved dose evaluation and recording procedures, utilizing multiple dosimeters on each individual to provide spectral data, or worker tracking systems to correlate with area monitor data should be implemented.
- 7-10. In order to reduce the uncertainty in the biological data upon which the evaluation of radiation risk must be based, the accuracy of conventional dosimetry must be improved. The goal of dosimetry for biological experiments should be to provide sufficient data on the radiation field and the irradiated subject that any needed dosimetric parameter could be determined for any point in the subject. Improved neutron and photon spectrometers and dosimeters are needed.
 - 11-14. Radiation protection records will probably provide the dosimetry for future epidemiological studies of radiation workers. If those future studies are to be as useful as possible, improvements need to be made in the accuracy and detail provided by radiation protection instrumentation.

CHAPTER V
CHEMICAL AND MOLECULAR MECHANISMS UNDERLYING CELLULAR RESPONSES

The primary energy deposition events from neutron irradiation are translated into biological/cellular damage through chemical and biochemical changes in cellular macromolecules and essential structures. Studies on low-LET radiation have provided insights into radiation chemical changes and stable structural changes in DNA and other macromolecules which have been implicated in the physiological responses to irradiation. An understanding of the LET dependence of these and other changes is needed with the aim of identifying changes qualitatively or quantitatively unique and indicative of neutron irradiation. Precise analyses may be facilitated by the use of well-defined charged particles, such as are produced by neutron recoils.

Research Needs	Comments
1. The dependence on neutron energy of the dose response for the production of reactive chemical species needs to be determined in concentrated aqueous media.	1. Comparative data are needed on the dose dependencies of the production of reactive species in aqueous media made to be representative of cellular protoplasm.
2. The dependence on neutron energy of the dose response for the production of chemical changes in DNA, RNA, proteins, and membranes should be elucidated.	2. Because of the likelihood that macromolecules contain the critical targets in cells, the dependence of the lesions registered in these molecules on the quality of the radiation should be determined.
3. A search should be undertaken to identify molecular lesions, and their yields, that are distinctively associated with neutron radiation, as opposed to low-LET radiation.	3. Because of the qualitative differences in the radiobiology of high- versus low-LET radiations, it is important to seek corresponding differences at a molecular level.
4. The chemical parameters which affect the radiochemical yields of the products of irradiation and their dependence on neutron energy should be determined.	4. The yield of various chemical and molecular effects in cells depends upon the radiochemical milieu. Hence, the influence of chemical modifiers on neutron effects needs to be examined.

5. The yield of DNA double strand breaks in cells, in the region of low doses, needs to be measured. How the yield depends on neutron energy, chromosomal location, dose protraction, and time after exposure needs to be determined.
6. Methods should be developed which will allow chemical and positional sub-classes of DNA double strand breaks, and other lesions, to be resolved.
7. Methods are required to permit the measurement of the dependence of clusters of lesions on neutron energy in cell-free systems which mimic the cellular milieu in comparison to cellular systems.
8. Information is needed on the dependence of lesions in critical molecules and cellular structures on neutron energy. These include the nuclear matrix, the nuclear lamina, the replication complex, and the nuclear membrane.
9. Information is needed on the influence of dose protraction on the nature of the lesions in critical molecules and structures, particularly the persistence of damage in such targets with time after irradiation, and on how such signals depend on neutron energy.
5. Various correlations suggest that DNA double strand breaks in cells may be important to end effects like cell killing, mutation, and neoplastic transformation. The dependence of such breaks on neutron quality, dose delivery, and cellular repair needs to be known.
6. The contribution of the several component lesions comprising the damage measured collectively as DNA double strand breaks may vary with LET.
7. Track structure may be an important LET-dependent feature of neutron irradiation. Methods are needed for measuring clusters of lesions in relevant molecules like DNA in plasmids treated extra- as well as intracellularly. Such measurements should be extended to genomic DNA in cells and tissues.
8. Because critical cellular targets may be structurally as well as molecularly complex, it is important to determine the dependence of damage in such targets on neutron energy. It would be expected that of principal concern would be the nuclear architecture of cells and the relationship of the genome and its organization to the nuclear machinery that is used to replicate and to segregate DNA.
9. In addition to low doses, the dependence of lesions produced in critical targets on repair/misrepair processes should be determined in order to facilitate a connection between molecular lesions and their phenotypic expressions.

10. Information is needed on the enzymatic competence of cells to recognize and to modify radiation-induced lesions and on how this ability depends on neutron energy.

10. In general, the phenotypic expression of radiation damage is influenced by enzymes that modify radiation-induced changes during or after the exposure. The quality or the quantity of lesions of a given type may depend on neutron energy and may influence the competence and/or the kinetics of the enzymes involved. Modulations such as these may offer important clues about the LET dependencies of different endpoints.

CHAPTER VI

CELLULAR AND GENETIC STUDIES

In reference to Chapter VI, *Cellular and Genetic Studies*, the needs for research fall into four broad categories with mutation and neoplastic transformation as the principal endpoints of interest. *First*, improvements are needed in the cell culture techniques for the assessment of hereditary change. *Second*, in light of the insights that have been forthcoming on oncogenes and antioncogenes in cancer, evidence is needed on the role of such genes in radiation-induced neoplastic transformation. *Third*, the systematics of the dependence of mutagenesis and neoplastic transformation on the species of origin is needed in order to enable the extrapolation of data from rodents to people. And *fourth*, information is needed on the dependence of mutagenesis and neoplastic transformation on neutron energy, that is, on neutron spectrum, and on other radiobiological parameters in order to develop an internally consistent understanding of the induction of these endpoints by radiation.

Research Needs	Comments
1. Additional lines of cells in culture—epithelial as well as fibroblast cells—to assess neoplastic transformation are needed. To be able to extrapolate from rodents to people, lines should be developed also from dog, monkey, and human tissues.	1. At the present, quantitative work on neoplastic transformation is largely limited to several lines of fibroblasts of rodent origin. More fibroblast lines are needed in order to ensure the generality of observations with fibroblasts. In addition, epithelial cell lines are needed because most human tumors arise in epithelium. In order to ensure the generality of observations across species, additional cell lines should be developed from dog, monkey, and human tissues, as well as from rodent material.
2. Improvements are needed in the media for the cultivation of cells that are used for the assessment of neoplastic transformation. Information is needed on the constituents of serum that are required for normal cell growth and for the phenotypic expression of unregulated growth.	2. In contemporary practice, serum at a level of 5-15% is used to supplement an otherwise fully defined medium. Serum contains a number of unidentified factors that can influence results and may vary from lot to lot. Developments are needed to replace currently-used media by those that will be completely defined and, therefore, be able to ensure reproducibility of results.

3. Data on the dependence of mutagenesis on neutron energy and other radiobiological parameters are needed, and molecular changes in radiation-induced mutants should be compared to spontaneous mutants.
4. Additional endpoints are needed to assess neoplastic transformation.
5. Evidence of radiation-induced changes in one or more oncogenes associated with, or responsible for, initiation, promotion, and/or progression are needed. Dependencies on neutron energy should be examined.
6. The dependence on radiobiological parameters of changes in oncogenes and antioncogenes has to be determined for cells of different histological types as well as for cells from rodent, dog, monkey, and human tissues.
3. A greater understanding is needed of the induction of heritable changes in specific genes. In addition to the dependence on neutron energy and the roles of repair/misrepair, molecular characterizations of the changes are needed.
4. At the present, focus formation in the growth of neoplastically transformed cells and altered morphology in the clonal growth of cells are the criteria that are usually applied. In addition to the foregoing, unregulated growth is associated with changes in other cellular properties, such as cellular growth control by autocrine pathways, defective communication, lifespan, surface antigens/receptor sites, internal receptor sites, and oncogene action.
5. Evidence of the activation of oncogenes or changes in antioncogenes is needed, and how such changes depend on radiobiological parameters such as neutron energy, dose rate, and cell repair. Further, genetic changes have to be identified that are responsible for initiation, promotion, and/or progression. If more than one genetic change is required, the dependence of each on radiobiological parameters and the role of repair/misrepair processes in their expression should be determined.
6. In order to effect an extrapolation to human beings, the systematics should be developed of how radiation-induced genetic changes depend upon the histology and the species of origin of the cells.

7. The chemical modification of radiation-induced mutation and neoplastic transformation has to be assessed. Also, similar information is needed with respect to other physical agents, such as ultraviolet light and ionizing radiation.
8. The qualitative and quantitative differences should be assessed between somatic mutations resulting from damage registered directly in a gene and its control elements—due to both direct and indirect radiation action—as opposed to changes resulting from action registered elsewhere in the genome. Radiation action directly in the gene may be random whereas mutations that result as a consequence of other action may not be. Differences in mechanisms of this type also should be examined as a function of LET, with fission-spectrum neutrons, and as a function of dose rate.
7. In addition to the roles of certain chemicals in promoting or suppressing the process of transformation of initiated cells, the possibility should be examined that directly or indirectly acting mutagens and oncogens may interact with radiation changes. Studies of this type are needed because they may help to understand the mechanism of radiation-induced heritable changes, but also because people are usually exposed to a chemical genotoxin(s) as well as to a physical one.
8. The evidence available supports the conclusion that intragenic mutations result from changes introduced at random into structural genes and their control elements. However, it is not known whether or not random intragenic changes would result if the mutation is a consequence of changes registered elsewhere in the genome. A possibly important mechanism in this connection is the induction of a loss of replicational fidelity as a result of which errors might be made in the gene in question that are not random. If this process occurs, it should be examined as a function of radiobiological parameters such as LET and dose rate.

CHAPTER VII
TISSUES AND ORGANS: DETERMINISTIC EFFECTS

Additional research on the relative biological effectiveness of neutrons of various energies with respect to dose-response relationships for deterministic effects is needed to provide the means to evaluate evolving theories about the relationship between effects at the level of the cell, organ, and animal. In addition, these data will strengthen radiation protection procedures and the effectiveness and safety of neutron and other high-LET radiation therapy.

Research Needs	Comments
1. Dependence of response on neutron energy, including energies in excess of 15 MeV, should be determined.	1-5. A systematic understanding of the effect of various parameters on RBE is needed. Data on each of the listed factors should be obtained for several representative endpoints. Endpoints which can be related to damage to specific types of cells should be emphasized.
2. Dependence of response on dose rate (fractionation/ protraction), including long-term irradiation, should be evaluated.	
3. Comparisons between mammalian species (including larger animals such as dogs and monkeys) to facilitate extrapolation of principles to man should be made.	
4. Comparison of effects of localized and whole body irradiation should be made.	
5. Relationships between normal cell kinetic parameters and the timing and extent of expression of the injury should be investigated.	

6. Studies to elucidate mechanisms of damage and recovery in the response of tissues and organs to long-term irradiation, including repair of sublethal and potentially lethal damage in cells, regeneration of functional tissue cells, and replacement of functional parenchymal cells by fibrous tissue, should be conducted.
 7. Increased effort to derive quantitative data from studies of radiotherapy patients and other human subjects exposed to neutron irradiation should be supported.
 8. Effects on the embryo and fetus irradiated *in utero* and the dependence of the response to irradiation after birth as a function of age or stage of development should be investigated further.
 9. Mathematical models which account for influence of long-term dose fractionation or protraction should be developed.
- 6-9. Additional work on the interpretation of experimental and epidemiological data is needed to clarify the underlying mechanisms and the implications of deterministic effects for predicting risks.

CHAPTER VIII STOCHASTIC EFFECTS

The stochastic effects of low doses are the primary concern in establishing radiation protection standards for neutrons. The basic outline of the stochastic effects has been established by previous research, but as the interpretation of these results progresses, gaps in the understanding of specific issues are revealed. A continuing research effort, guided by the analysis of past results, is needed to reduce the uncertainties in extrapolating from experimental results in animals to risks for man.

Research Needs	Comments
1. Complete and precise dosimetric data should be assured through adherence to dosimetry protocols, participation in intercomparisons, and site visits to identify preferred techniques.	1. A complete characterization of the radiation field and exposure geometry is needed to provide data on dose and radiation quality to the various tissues of the exposed animal.
2. Analysis of data on <i>in vivo</i> carcinogenesis by actuarial methods and different mathematical models, for example, the proportional hazards model or analytical models such as Weibull distribution, should be performed.	2. Large numbers of competing effects and dependence on a variety of biological and physical variables have to be taken into account.
3. New experiments on <i>in vivo</i> carcinogenesis as a function of dose rate or multiple fractionation schedule for low- and high-LET radiation should be initiated.	3. Modern analysis of previously obtained data shows gaps which are critical to the understanding of tissue and species dependent effects.
4. Long-term epidemiological studies on radiation workers and cancer patients treated with external beams of fast neutrons should be continued.	4. Epidemiological data are the only source of information to test the connection between animal studies and dose-response models for human risks.
5. The RBE _M for dominant mutations and changes in activity of enzymes in mouse spermatogonia should be studied at low dose levels.	5. Endpoints where neutron effects at the doses of concern in radiation protection can be studied are needed to test the validity of extrapolation to those low doses.

6. Full use should be made of available specimens from persons exposed to neutrons either accidentally or radiotherapeutically for the purpose of studying chromosomal defects in peripheral lymphocytes and germ cells.
7. The nature of radiation-induced dominant and recessive mutations at defined gene loci should be investigated.
6. All sources of human data should be utilized.
7. Information of importance in elucidating mechanisms of genetic effects can be derived from studies of well-characterized mutations with newer molecular tools.

Chapter X

INTEGRATED APPROACH TO EXAMINING THE BIOLOGICAL EFFECTIVENESS OF NEUTRONS*

The integrated approach proposed here consists of recommendations for additional research for specific research areas and objectives. These recommendations are meant to reflect not only the importance of the anticipated research results to the desired improvement in estimation of human health risks from exposure to neutrons, but also the general aspects of anticipated timeliness of technological progress and research feasibility as the recommended research plan progresses.

ESTIMATE CANCER RISKS IN HUMAN POPULATIONS EXPOSED TO NEUTRONS

Since the ultimate goal of the proposed research program is to improve the estimate of human cancer risk from neutrons, valuable information may be available from studies of those human populations now known to have been exposed to neutrons (e.g., neutron radiotherapy patients and workers at nuclear facilities) and other populations as they may be identified. Every new primary tumor should be documented and a complete data base maintained for these populations. Such sets of data will probably be limited because of the availability of exposed populations, inadequate dosimetry, absence of suitable controls, limited ranges of dose and dose rate, varying degrees of follow-up, and other confounding variables. In spite of these limitations, useful information on cancer incidence will accrue within the short term and continue to be forthcoming with further testing of models, even purely phenomenological models.

Use of these populations will have a greater impact to the extent that they can be a resource to test mechanisms, based on developing knowledge, on how neutron irradiation causes cancer. Therefore, wherever possible, blood and tissue samples should be examined by cytogenetic and molecular genetic procedures, and tumor and normal tissue samples should be analyzed by molecular genetic techniques in order to document specific oncogenic changes suspected on the basis of mechanistic studies to play a causative role in the tumor type under study. The samples should be catalogued and stored for future examination. Epidemiological studies are valuable both for the insights they provide with respect to the estimates of risk for the development of human cancer, as well as for the directions they offer for mechanistic studies. The iterative process between the epidemiological approaches and the molecular approaches will strengthen the usefulness of both.

* The major part of the material in this report was prepared prior to June 1991.

EXTRAPOLATE HUMAN CANCER RISK FOR NEUTRONS FROM ANIMAL DATA

The concept of a neutron RBE is based on the comparative effectiveness of neutrons versus that of low-LET radiation, notably gamma rays or x rays. For some endpoints in animals, the effectiveness of low-LET (reference) radiation at low doses is vanishingly small. The consequent extremely high RBE values would indicate an erroneous overestimate of the danger of neutrons. It is important to obtain information on the risk per unit dose value for both types of radiation. It is particularly relevant to ensure that the dose response curves selected for animal studies reflect the same basic functional relationships as observed in man.

Results of studies on tumorigenesis in experimental animals are of value for the following purposes:

- (1) extrapolation to the low dose and low dose rate regions of both low- and high-LET radiations;
- (2) estimation of the effectiveness of both types of radiation;
- (3) discovery of possible differences in histopathological characteristics of the induced malignancies as a function of LET; and
- (4) provision of well-characterized tissue samples for studies of mechanisms of tumorigenesis.

In view of the increasing difficulties in obtaining funding for large-scale *in vivo* animal experiments, some priorities are proposed.

(1) Valuable information could evolve from the wealth of tissue samples which have been collected during previous experiments but have not been analyzed because they were peripheral to the original study, the required techniques were not yet available, or there was a lack of time or funds. Priority should be given to an organized effort to collect and evaluate this information from completed studies. In Europe, such a project has been initiated under the sponsorship of the European Late Effects Project Group (EULEP).

(2) In order to provide relevant extrapolation information between animal data and existing human data, it is important to compare the shapes of the dose response relationships for comparable endpoints.

(3) *In vitro* cellular and molecular biology methodology could provide guidance for a limited selection of animal studies aimed at elucidating specific problems. The discovery of specific genes associated with certain late effects would always require confirmation from an *in vivo* experiment.

(4) The phenomenon of the inverse dose rate effect should be further investigated by a selected number of *in vivo* tumorigenic studies using different species and dose rates. As an example, priority could be placed on the induction of specific epithelial type of tumors as an addition to the already existing information on radiation-induced myeloid leukemia.

IMPROVE HUMAN CANCER RISK ESTIMATES THROUGH UNDERSTANDING OF MECHANISMS

Due to the limitations of human data in terms of statistics and types of exposures where data are available, and the limitations of extrapolating animal data to humans, the long term solution to estimating health risks will depend on a better understanding of the underlying mechanisms. Although extensive progress has been made in understanding some of the steps in radiation-induced neoplastic transformation, gaps remain in our knowledge of the steps between irradiation and tumor formation. These include: (1) the identification of the molecular changes in cells that initiate the transformation process; (2) the mechanisms for the induction of somatic mutations that promote transformation; and (3) the identification of the specific oncogenes causally related to radiation transformation. For neutron irradiation, the information is even more limited. The research plan described here is designed to reduce the gaps in our knowledge of radiation-induced transformation. It depends on the application of modern molecular techniques for detailed studies in *in vitro* systems and on integrating the research with the programs recommended above, so that the *in vivo* and *in vitro* studies complement one another.

The remaining problem in evaluating risks at doses and dose rates encountered in the environment is the extrapolation from data obtained at much higher levels. At these environmental levels neutron exposures are dominated by the interaction of single charged particles with individual cells. Thus, evaluation of the biomolecular and cellular effects of irradiation by single charged particles is required.

(1) Mechanisms of neutron-induced neoplastic transformation.

(a) The dependence of neutron-induced neoplastic transformation on changes in oncogenes and suppressor genes, and on their expression, needs to be determined in order to establish which specific genetic alterations are important in radiation-induced neoplastic transformation and how this depends on radiation quality.

(b) A more complete understanding is needed of the mechanisms by which neutron-induced chemical changes in cellular structures lead to neoplastic transformation and how these changes depend, either qualitatively or quantitatively, on radiation quality. Changes in DNA should be identified which would have a high likelihood of being causative factors in cellular response to neutrons, as compared to low-LET radiation. There is a need to develop the methodology so that biochemical measurements can be extended to lower doses.

(c) Mechanisms by which radiation damage to genomic structures is repaired or metabolically altered to result in heritable changes in cells need to be determined, as well as how these may depend upon radiation quality and dose protraction.

(d) The neutron-induced genetic changes which give rise to mutation and transformation should be determined at the level of gene mapping and DNA sequence. Mechanisms of mutagenesis initiated by radiation need to be elucidated with emphasis on the dependence of specific types of mutations on dose, dose rate, and radiation quality.

(e) The oncogenes and suppressor genes responsible for specific malignancies and the role of tissue and species differences in determining these oncogenes should be investigated. The aims should be: (i) to distinguish modes of involvement of oncogenes identified by the studies described in cells of epithelial origin, from which most human tumors derive, in comparison to other tissues; and (ii) to delineate commonalities and differences in mechanisms in similar tissues derived from different species.

(2) Extrapolation to effects of low dose rate and high LET.

(a) Experiments should be performed to evaluate chromosome aberration frequency and neoplastic transformation for cultured cell types *in vitro* exposed to single charged particles, such as might be produced as neutron secondaries. The effect of time between charged particle tracks traversing individual cells should be investigated for the above endpoints.

(b) Single cell assay techniques capable of resolving early stages of damage in cells irradiated by single charged particles should be developed. These techniques might include determination of induced breaks in prematurely condensed chromosomes and changes in chromatin structure or alterations in specific gene expression in single cells.

(c) Methods should be developed to study the consequences of single cells traversed by a charged particle in an organized multicellular structure in culture.

FACILITIES, RESOURCES, AND ENVIRONMENT

An effective program focused upon the radiobiology of neutrons requires that adequate laboratory and irradiation facilities are available and adequately staffed. These requirements should include sources of well-characterized fission-spectrum neutrons and monoenergetic particles. These facilities should be designed to enable: high- and low-dose-rate exposures; low-dose protraction over days and months; the exposure of cells in culture vessels under controlled conditions of temperature (0° - 37° C) and gaseous atmosphere; and the irradiation of animals.

Because of the need to integrate studies from molecules to cells, tissues, and animals, it is also required that the intellectual environment for this program should include a corresponding range of scientific interests and talents.

(1) To insure that the data obtained at different radiation facilities are comparable, dosimetry intercomparisons should be made. These should include spectral analyses of neutron beams and characterization of their gamma-ray contaminations.

(2) A facility for these studies should also be exploited for the purpose of postdoctoral training of young scientists and predoctoral thesis research, as may be possible.

(3) Provisions must be made for cataloging and storing samples from exposed humans and animals, so that new molecular techniques can be applied as they are developed. Also, a catalog of past experiments should be made available so that results can be utilized in evaluating new hypotheses as they are developed.

(4) Continuity of funding is essential in order to accomplish the above goals.

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APPENDIX A

CIRRPC SCIENCE SUBPANEL ON HIGH-LET RADIATION

Recognition should be given to the following individuals and the agencies they represented at the time of their service on the Subpanel.

Dr. Bruce W. Wachholz, Chairman
National Institutes of Health
U.S. Department of Health and Human Services

Mr. J. Joseph Coyne
National Bureau of Standards
U.S. Department of Commerce

CDR Eric Kearsley, MSC, USN
Armed Forces Radiobiology Research Institute
U.S. Department of Defense

Dr. Lawrence S. Myers, Jr.
Armed Forces Radiobiology Research Institute
U.S. Department of Defense

Dr. D. Stuart Nachtwey
Johnson Space Center
U.S. National Aeronautics and Space Administration

CDR Gary Zeman
Armed Forces Radiobiology Research Institute
U.S. Department of Defense