

Fifth Edition

# Radiobiology for the Radiologist

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## Preface to the First Edition

This book, like so many before it, grew out of a set of lecture notes. The lectures were given during the autumn months of 1969, 1970, and 1971 at the Columbia-Presbyterian Medical Center, New York City. The audience consisted primarily of radiology residents from Columbia, affiliated schools and hospitals, and various other institutions in and around the city.

To plan a course in radiobiology involves a choice between, on the one hand, dealing at length and in depth with those few areas of the subject in which one has personal expertise as an experimenter or, on the other hand, surveying the whole field of interest to the radiologist, necessarily in less depth. The former course is very much simpler for the lecturer and in many ways more satisfying; it is, however, of very little use to the aspiring radiologist who, if this course is followed, learns too much about too little and fails to get an overall picture of radiobiology. Consequently, I opted in the original lectures, and now in this book, to cover the whole field of radiobiology as it pertains to radiology. I have endeavored to avoid becoming evangelical over those areas of the subject which interest me, those to which I have devoted a great deal of my life. At the same time I have attempted to cover, with as much enthusiasm as I could muster and from as much knowledge as I could glean, those areas in which I had no particular expertise or personal experience.

This book, then, was conceived and written for the radiologist—specifically, the radiologist who, motivated ideally by an inquiring mind or more realistically by the need to pass an examination, elects to study the biological foundations of radiology. It may incidentally serve also as a text for graduate students in the life sciences or even as a review of radiobiology for active researchers whose viewpoint has been restricted to their own area of interest. If the book serves these functions, too, the author is doubly happy, but first and foremost it is intended as a didactic text for the student of radiology.

Radiology is not a homogenous discipline. The diagnostician and therapist have divergent interests; indeed it sometimes seems that they come together only when history and convenience dictate that they share a common course in physics or radiobiology. The bulk of this book will be of concern, and hopefully of interest, to all radiologists. The diagnostic radiologist is commended particularly to Chapters 11, 12, and 13 concerning radiation accidents, late effects, and the irradiation of the embryo and fetus. A few chapters, particularly Chapters 8, 9, 15, and 16, are so specifically oriented towards radiotherapy that the diagnostician may omit them without loss of continuity.

A word concerning reference material is in order. The ideas contained in this book represent, in the author's estimate, the consensus of opinion as expressed in the scientific literature. For ease of reading, the text has not been broken up with a large number of direct references. Instead a selection of general references has been included at the end of each chapter for the reader who wishes to pursue the subject further.

I wish to record the lasting debt that I owe my former colleagues at Oxford and my present colleagues at Columbia, for it is in the daily cut and thrust of debate and discussion that ideas are formulated and views tested.

Finally, I would like to thank the young men and women who have regularly attended my classes. Their inquiring minds have forced me to study hard and reflect carefully before facing them in a lecture room. As each group of students has grown in maturity and understanding, I have experienced a teacher's satisfaction and joy in the belief that their growth was due in some small measure to my efforts.

E.J.H.

New York

July 1972

## Preface

This fifth edition has been completely revised and substantially rewritten. The format has been changed so that Part 1, the first 15 chapters, represents both a general introduction to radiation biology and a complete self-contained course in the subject, suitable for residents in diagnostic radiology and nuclear medicine. It follows the format of the Syllabus in Radiation Biology prepared by the Radiological Society of North America (RSNA) in 1999, and its content reflects the questions appearing in recent years in the written examination for diagnostic radiology residents of the American Board of Radiology.

Part 2 consists of more in-depth material designed primarily for residents in radiation oncology. It begins with a chapter on molecular techniques, followed by a chapter on cancer biology, new to this edition.

Times change, fashions are modified, and the relative emphasis of different topics must be revised. "New" radiation modalities become "alternative" radiation modalities, and this chapter is considerably abbreviated because neutrons and heavy ions have not lived up to their earlier promise, while pions have disappeared altogether. Hypoxic cell radiosensitizers give way to hypoxic cytotoxins, formerly termed bioreductive drugs. Predictive assays are still a tantalizing dream, but may never really come into their own until molecular and genetic assays replace measurements of cellular sensitivity.

A new chapter in this edition describes the basis of gene therapy, an exciting topic that has enough promise to be worth a mention. A frequent criticism of the third and fourth editions of this book was the absence of a chapter on radiation effects in normal tissues in humans. This absence in the past reflected my own personal ignorance and lack of expertise in the area, as well as the conviction that this was clinical radiation oncology, with no place in a text on radiation biology. However, since no simple summary of this subject exists, I have yielded to the pressure to include a chapter on normal tissue effects, gleaning basic information from many sources, and seeking advice and counsel from my clinical colleagues.

Most of the other chapters have simply been revised and updated to reflect current thoughts and ideas. For example, we now see hypoxia not simply as a modifier of radiation response, but as an element of the tumor microenvironment driving aggression and malignancy. The cell cycle, too, is no longer an empirical series of events, but is driven by cyclines and cyclin-dependent kinases.

This fifth edition is certainly the last. This prediction can be made with some confidence because I feel that the days of the single-authored text are numbered, if not over! A book written entirely by one person has the advantage of continuity of style and depth of coverage. However, it is increasingly difficult for any one person to keep up with a rapidly expanding field, as well as with the introduction of molecular techniques. A series of chapters, each written by the expert in that area, may be somewhat daunting to the new student, but in the end it ensures accuracy and an up-to-date account.

I feel a lasting debt to the young men and women who have attended my lectures at Columbia-Presbyterian, as well as my refresher courses at ASTRO and RSNA over a period of 30 years. Their sharp and enquiring minds have forced me to keep up to date, while their need to digest an ever-expanding field of molecular and cellular radiobiology, while concentrating on patient care, has taught me to be brief and to distill out the essential facts.

*Eric J. Hall, D.Phil., D.Sc., F.A.C.R., F.R.C.R.*

## Acknowledgments

I would like to thank the many friends and colleagues who generously and willingly gave permission for diagrams and illustrations from their published work to be reproduced in this book.

While the ultimate responsibility for the content of this book must be mine, I acknowledge with gratitude the help of a number of friends who read chapters relating to their own areas of special expertise and made invaluable suggestions and additions. With each successive edition, this list grows longer, and now includes Drs. Ged Adams, Philip Alderson, Sally Amundsen, Joel Bedford, Roger Berry, Max Boone, Victor Bond, J. Martin Brown, Ed Bump, Julie Choi, Bill Dewey, Frank Ellis, Peter Esser, Stan Field, Greg Fryer, Charles Geard, Eugene Gerner, Amato Giaccia, Julian Gibbs, George Hahn, Simon Hall, Tom Hei, Robert Kallman, Howard Lieberman, Philip Lorio, Edmund Malaise, Gillies McKenna, Mortimer Mendelsohn, George Merriam, Noelle Metting, Jim Mitchell, Anthony Nias, Ray Oliver, Julian Preston, Elaine Ron, Harald Rossi, Robert Rugh, Robert Sutherland, Roy Tishler, Len Tolmach, Liz Travis, Lou Wagner, John Ward, Barry Winston, Rod Withers, Basil Worgul, Stanley Order, Dennis Leeper, and James Cox. Without their help this volume would be much the poorer. Most of the original illustrations in this book were created by Brian Soda, and I am glad of this opportunity to applaud his skill.

The principal credit for this book must go to the successive classes of residents in radiology, radiation oncology, and nuclear medicine that I have taught at Columbia and at ASTRO and RSNA refresher courses over a period of more than 30 years. Their perceptive minds and searching questions have kept me on my toes. Their impatience to learn what was needed of radiobiology, and to get on with being doctors, has continually prompted me to summarize and get to the point!

I am deeply indebted to the United States Department of Energy, the National Cancer Institute, and the National Aeronautical and Space Administration, who have generously supported my work, and indeed much of the research performed by numerous investigators, that is described in this book.

I owe an enormous debt of gratitude to Michaela Delegianis, who not only typed and formatted all of the chapters, but played a major role in proofreading and editing.

Finally, I thank my wife, Bernice, who has been most patient and gave me every encouragement when I needed it most. She also spent many hours proofreading the manuscript.



Fifth Edition

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Radiobiology  
for the Radiologist

Philip Edition

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for the Radiologist

Philip D. Phillips, M.D.

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# Milestones in the Radiation Sciences

Now that the centennials of all of the major events involved with the genesis of both diagnostic radiology and radiation oncology have well and truly passed, it seems appropriate to compile a list of "milestones" of the principal events that have brought us to where we are today. The principal motive for doing so is that we need the constant reminder that each generation stands on the shoulders of the one that went before — or, as Sylvanus Thompson, the first president of the Roentgen Society, put it more eloquently soon after the discovery of x-rays:

In the history of Science, nothing is more true than that the discoverer, even the greatest discoverer, is but the descendant of his scientific fore-fathers; he is always essentially the product of the age in which he is born.

1859—Darwin: Changes in populations of organisms.

1865—Mendel: Traits inherited by individual organisms.

1895—Röentgen discovered x-rays.

1896—Becquerel presented to the Paris Academy of Sciences the results of his discovery of radiations emitted by uranium compounds.

1896—First biologic effects of x-rays reported included skin "burns," epilation, and eye irritation.

1896—Treatment of a hairy nevus by Freud.

1897—Rutherford examined the radiations from uranium after Becquerel's discovery of radioactivity and found two types, which he

called α- and β-rays. Later he found that α-particles consist of nuclei of helium and that β-particles consist of electrons discovered by Thomson.

—Grubbe, Despeignes, Williams, Voigt; Rival claims of first use of x-rays to treat cancer.

1898—Marie and Pierre Curie announced the discovery of "polonium" in July and of "radium" in December.

1902—Cancer in x-ray ulcer reported: Frieben.

1903—"Law" of Bergonie and Tribondeau; radiosensitivity related to mitotic activity.

—First suggestion to treat cancer by implanting radium: Bell.

1905—Chromosome theory of heredity.

1911—Leukemia in five radiation workers reported: Jagic.

1913—Bohr suggested a model of the atom with a central nucleus and electrons moving in orbits around it.

—Coolidge built the first successful roentgen-ray tube with hot filament and tungsten target.

1915—British Roentgen Society introduced proposals for radiation protection.

1919—Rutherford bombarded nitrogen atoms with α-particles and found that the nuclei of these atoms disintegrated, giving off hydrogen; oxygen atoms were left. The particles given off were found to be positively charged, and Rutherford named **them protons**. This was the first experiment in which one element was transformed artificially into another element, namely nitrogen into oxygen.

1922—Compton discovered the "Compton effect," namely the change in wavelength of scattered x-rays.

1923—Eugene Petry discovered the oxygen effect with plant roots.

1927—Rabbit testes experiments suggested the value of fractionation in radiotherapy: Regaud and Ferraux.

—First observation of mutations by x-rays in Drosophila: Müller.

1928—Wilderöe suggested the principle of the cyclotron.

—Coutard reported superiority of fractionated treatment for human cancer.

—Unit of x-ray intensity proposed by Second International Congress of Radiology.

—International Committee on X-ray and Radium Protection established.

—First international recommendations on radiation protection adopted by Second International Congress of Radiology.

1929—Advisory Committee on X-ray and Radium Protection established (United States).

1930—Lea: First survival curve for bacteria exposed to radiation.

1931—The roentgen adopted as the unit of exposure for x-radiation.

1932—Lawrence invented the cyclotron. In 1933, collaborating with M. S. Livingston, he built a cyclotron capable of producing 5,000,000-V deuterons.

—Chadwick announced the discovery of the neutron, a neutral nuclear particle of about the same mass as the positively charged proton. This experimental proof of the existence of the neutron confirmed speculations made by Rutherford in 1919.

1933—Crabtree and Cramer: Oxygen affects radiosensitivity of tumor "slices"; postulated importance of oxygen in radiotherapy.

1934—Joliot and Irene Joliot-Curie produced artificial radioactivity by bombarding aluminum with  $\alpha$ -particles and observed that neutrons and positively charged particles were emitted from the aluminum during this process.

—Paterson and Parker introduced their dosage system for y-ray therapy.

1935—Mottram noted the effect of oxygen on radiosensitivity of *Vicia faba* roots and postulated its importance to radiotherapy.

1937—The Fifth International Congress of Radiology accepted the roentgen as an international dosage unit for x- and y-radiation.

1938-39—**37-inch** cyclotron at Berkeley used to treat first patient with neutrons by Robert Stone.

1940—Lea and Catcheside proposed the linear-quadratic formalism for biologic response to radiation.

—First quantitative oxygen enhancement ratio measured by Gray; published 1952.

—Zirke introduced the concept of linear energy transfer.

1941—The principle of "one gene-one enzyme" established.

1942—First self-maintaining nuclear chain reaction in a uranium graphite pile or reactor was initiated in Chicago: Fermi and colleagues.

1943—First use of radioactive isotopes to label compounds in biology and medicine: Hévesy.

1944—Strandquist: Relation between dose and overall time for skin reaction proposed dose  $a(t/\text{time})^{0.33}$ .

1945—Atomic bombs exploded on July 16 in New Mexico, August 6 in Hiroshima, and August 11 in Nagasaki.

1946—Advisory Committee on X-ray and Radium Protection reorganized to the National Committee on Radiation Protection (United States).

1949—Discovery of cysteine as a radioprotector: Patt.

1950—International Commission on Radiological Protection and International Commission on Radiological Units reorganized from prewar committees.

—Erwin Chargaff discovered a consistent one-to-one ratio of adenine to thymine and guanine to cytosine in DNA.

1951—First clinical cobalt-60 unit, London, Ontario, Canada.

- Hereditary effects of radiation in mice reported: Russell.
- First patient treated with boron neutron capture therapy: Sweet.
- Linus Pauling obtained precise measurements of a helical polypeptide structure.
- 1952—First quantitative measurement of the oxygen effect published: Gray. <sub>sv</sub>
- DNA identified as the molecule of heredity.
- 1953—International Commission on Radiological Units introduced concept of absorbed dose.
- 1953—Development of autoradiography and elucidation of the phases of the cell cycle: Howard and Pelc.
- 1953—First linear accelerator to treat patients, Hammersmith Hospital, United Kingdom.
- 1953—Structure of DNA discovered: Crick and Watson.
- 1954—Indium-192 introduced into brachytherapy.
- 1955—Chronic hypoxia resulting from limitation of oxygen diffusion described: Thomlinson and Gray.
- 1956—The first *in vitro* radiation survival curve for mammalian cells: Puck.
- 1957—The K-curve for oxygen published: Howard-Flanders and Alper.
- 1959—Repair demonstrated by split dose experiment with mammalian cells: Elkind.
- First *in vivo* survival curve for tumor cells: Hewitt and Wilson.
- 1960—Survival curve shape change with linear energy transfer: Barendsen and colleagues.
- Concept of growth fraction in tumors: Mendelsohn.
- 1961—Remote afterloading for brachytherapy: Henscke.
- 1962—First demonstration of the dose-rate effect in cells *in vitro*: Hall and Bedford.
- 1963—Relation between electron affinity and radiosensitizing potential: Adams and Dewey.
- First observation of variation of radiosensitivity through the cell cycle: Terasima and Tolmach.
- First demonstration that hypoxic cells limit curability of a mouse tumor by x-rays; Powers and Tolmach.
- 1966—Potentially lethal damage repair described: Tolmach.
- First patient treated in hyperbaric oxygen: Churchill Davidson.
- Genetic code solved.
- Dependence of oxygen enhancement ratio on linear energy transfer: Barendsen and colleagues.
- 1967—Concept of cell loss factor in tumors: Steel.
- First survival curve for cells *in vivo*—skin colonies: Withers.
- 1968—Classification of tissue radiosensitivity: Casarett.
- Description of the nominal standard dose system: Ellis.
- 1969—Accelerated repopulation shown in animal tumors: Hermens and Barendsen.
- 1971—First cell-survival curves for hyperthermia.
- Development of assay for crypt cells in mouse jejunum: Withers.
- Survival curve for bone marrow stem cells: Till and McCulloch.
- Sensitivity to heat through the cell cycle: Westra and Dewey.
- Two-hit model to explain the paradigm of retinoblastoma: Knudsen.
- 1972—First computed tomographic scanner by EMI installed in a hospital in London.
- First recombinant DNA molecules produced.
- The term *reoxygenation* coined by Kailman.
- 1973—Time course of proliferation in normal tissues following irradiation: Denekamp.
- 1974—First clinical trial with neutrons: Catterall.
- First cancer patients treated with negative 7*i*-mesons at Los Alamos: Kligerman.
- 1975—First cancer patients treated with heavy ions at Berkeley.
- 1976—Fowler and Douglas derive linear-quadratic parameters from fractionation experiments.

- First randomized clinical trial of neutrons, Hammersmith Hospital.
- Development of spheroids: Sutherland.
- First clinical trial of a hypoxic cell radiosensitizer (metronidazole): Urtason and colleagues.
- Suppressor genes described in cultured cells: Stanbridge.
- 1979—Acutely hypoxic cells described: Brown.
- 1980—Difference in survival curve shape for early- and late-responding tissues: Withers.
- First repair gene in human cells: Rubin.
- First description of apoptosis: Kerr.
- First commercial magnetic resonance unit.
- 1981—Estimation of hereditary effects of radiation in humans: Schull, Otaka, Neel.
- 1982—Concept of biologically effective dose described: Barendsen.
- The first human oncogenes described: Bishop.
- 1985—First computer-controlled afterloader: Nucletron.
- Estimation of  $T_{pot}$  (potential doubling time) in patients from a single biopsy: Begg.
- 1986—Development of bioreductive drugs: Brown, Adams.
- 1989—Measurement of oxygenation status in human tumors with labeled nitroimidazoles: Chapman, Urtason, and colleagues.
- Polymerase chain reaction developed.
- 1990—Discovery of importance of mismatch repair genes in human colon cancer: Vogelstein.
- 1991—Single-strand conformal polymorphism technique developed to detect mutations.
- First use of gene therapy in animals.
- First correlation of SF2 (surviving fractions at 2 Gy) and tumor control: West.
- 1992—First clinical trial of WR2721 as a radioprotector: Kligerman.
- 1995—ATM gene sequenced.
- 1996—p53 named as the molecule of the year—the guardian of the genome.

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# The Physics and Chemistry of Radiation Absorption

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TYPES OF IONIZING RADIATIONS  
ABSORPTION OF X-RAYS  
DIRECT AND INDIRECT ACTION OF  
RADIATION

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ABSORPTION OF NEUTRONS  
CONTRAST BETWEEN NEUTRONS AND  
PHOTONS  
SUMMARY OF PERTINENT CONCLUSIONS

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In 1895 the German physicist Wilhelm Conrad Röentgen discovered "a new kind of ray," emitted by a gas discharge tube, that could blacken photographic film contained in light-tight containers. He called these rays x-rays, in his first announcement in December 1895—the x representing the unknown. In demonstrating the properties of x-rays at a public lecture, Röentgen asked Rudolf Albert van Kölliker, a prominent Swiss professor of anatomy, to put his hand in the beam and so produced the first radiograph (Fig. 1.1). The first medical use of x-rays was reported in the *Lancet* of January 23, 1896. In this report, x-rays were used to locate a piece of a knife in the backbone of a drunken sailor, who was paralyzed until the fragment was removed following its localization. The new technology spread rapidly through Europe and the United States, and the field of diagnostic radiology was born. There is some debate about who first used x-rays therapeutically, but by 1897, Wilhelm Alexander Freud, a German surgeon, demonstrated before the Vienna Medical Society the disappearance of a hairy mole following treatment with x-rays. Antoine Henri Becquerel discovered radioactivity in 1898, and radium

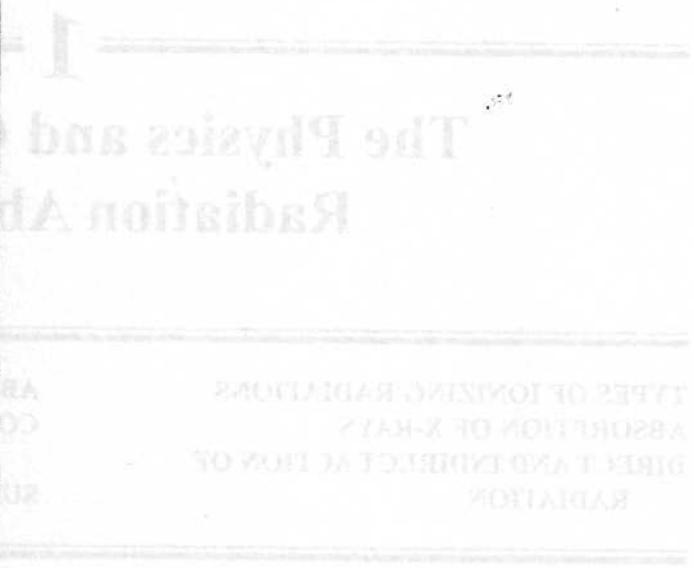
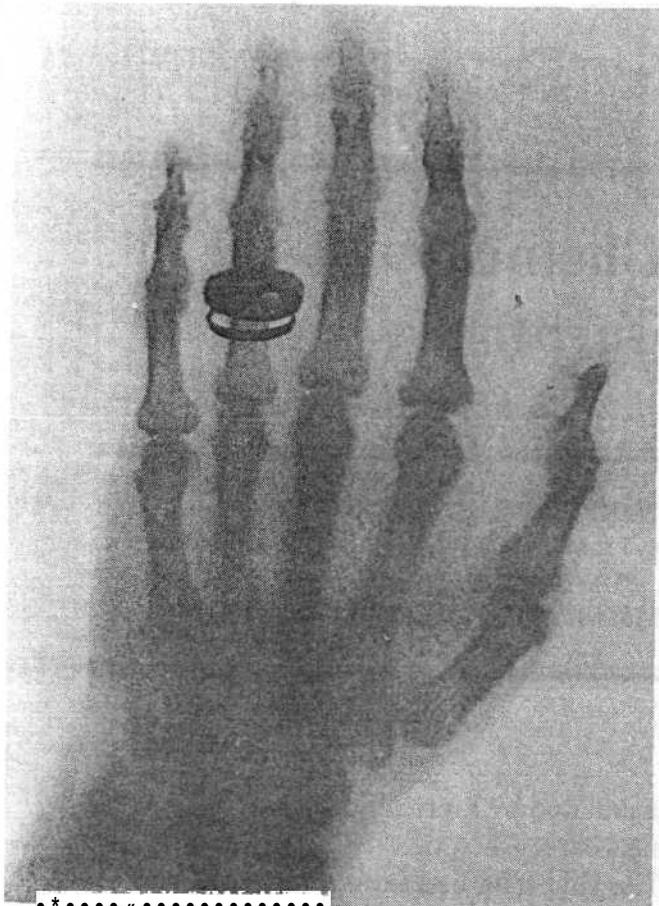
was isolated by Pierre and Marie Curie in the same year.

The first recorded experiment in radiobiology was performed by Becquerel when he inadvertently left a radium container in his vest pocket. He subsequently described the skin erythema that appeared 2 weeks later and the ulceration that developed and required several weeks to heal. It is said that Pierre Curie repeated the experiment in 1901 by deliberately producing a radium "burn" on his own forearm (Fig. 1.2). From these early beginnings, just before the turn of the century, the study of radiobiology began.

**Radiobiology** is the study of the action of ionizing radiations on living things. As such, it inevitably involves a certain amount of radiation physics. The purpose of this chapter is to present, in summary form and with a minimum of mathematics, a listing of the various types of ionizing radiations and a description of the physics and chemistry of the processes by which radiation is absorbed.

## **TYPES OF IONIZING RADIATIONS**

The absorption of energy from radiation in biologic material may lead to *excitation* or to



**Figure 1.1.** The first radiograph of a living object, taken in January 1896, just a few months after the discovery of x-rays. (Courtesy of Röntgen Museum, Wurzburg, Germany)



**Figure 1.2.** The first radiobiology experiment. Pierre Curie is said to have used a radium tube to produce a radiation ulcer on his arm. He charted its appearance and subsequent healing.

**ionization.** The raising of an electron in an atom or molecule to a higher energy level without actual ejection of the electron is called **excitation.** If the radiation has sufficient energy to eject one or more orbital electrons from the atom or molecule, the process is called **ionization**, and that radiation is said to be ionizing radiation. The important characteristic of ionizing radiation is the localized release of large amounts of energy. The energy dissipated per ionizing event is about 33 eV, which is more than enough to break a strong chemical bond; for example, the energy associated with a C=C bond is 4.9 eV. For convenience it is usual to classify ionizing radiations as **electromagnetic** or **particulate**.

### Electromagnetic Radiations

Most experiments with biologic systems have involved x- or y-rays, two forms of electromagnetic radiation. X- and y-rays do not differ in nature or in properties; the designations x- or y-rays reflects the way in which they are produced. X-rays are produced *extranuclearly*; y-rays are produced *intranuclearly*. In practical terms this means that x-rays are produced in an electrical device that accelerates electrons to high energy and then stops them abruptly in a target, usually made of tungsten or gold. Part of the kinetic energy (the energy of motion) of the electrons is converted into x-rays. On the other hand, y-rays are emitted by radioactive isotopes; they represent excess energy that is given off as the unstable nucleus breaks up and decays in its efforts to reach a stable form. Natural background radiation from rocks in the earth also includes y-rays. Everything that is stated of x-rays in this chapter applies equally well to y-rays.

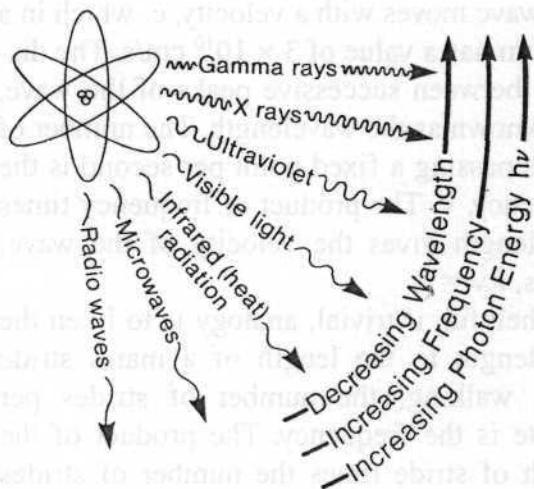
X-rays may be considered from two different standpoints. First, they may be thought of as waves of electrical and magnetic energy. The magnetic and electrical fields, in planes at right angles to one another, vary with time so that the wave moves forward in much the same way as ripples move over the surface of a pond if a stone is dropped into the water.

The wave moves with a velocity,  $c$ , which in a vacuum has a value of  $3 \times 10^{10}$  cm/s. The distance between successive peaks of the wave,  $X$ , is known as the wavelength. The number of waves passing a fixed point per second is the frequency,  $v$ . The product of frequency times wavelength gives the velocity of the wave; that is,  $Xv = c$ .

A helpful, if trivial, analogy is to liken the wavelength to the length of a man's stride when walking; the number of strides per minute is the frequency. The product of the length of stride times the number of strides per minute then gives the speed, or velocity, of the walker.

Like x-rays, radio waves, radar, radiant heat, and visible light are forms of electromagnetic radiation. They all have the same velocity,  $c$ , but they have different wavelengths and therefore different frequencies. To extend the previous analogy, different radiations may be likened to a group of men, some tall, some short, walking together at the same speed. The tall men take long measured strides but make few strides per minute; to keep up, the short men compensate for the shortness of their strides by increasing the frequencies of their strides. A radio wave may have a distance between successive peaks (*i.e.*, wavelength) of 300 m; for a wave of visible light the corresponding distance is about five hundred thousandths of a centimeter ( $5 \times 10^{-5}$  cm). The wavelength for x-rays may be one hundred millionth of a centimeter ( $10^{-8}$  cm). X- and y-rays, then, occupy the short-wavelength end of the electromagnetic spectrum (Fig. 1.3).

Alternatively, x-rays may be thought of as streams of photons, or "packets" of energy. Each energy packet contains an amount of energy equal to  $hv$ , where  $h$  is known as Planck's constant and  $v$  is the frequency. If a radiation has a long wavelength, it has a small frequency, and so the energy per photon is small. Conversely, if a given radiation has a short wavelength, the frequency is large and the energy per photon is large. There is a simple numeric relationship between the photon



**Figure 1.3.** Illustration of the electromagnetic spectrum. X-rays and y-rays have the same nature as visible light, radiant heat, and radio waves; however, they have shorter wavelengths and consequently a larger photon energy. As a result, x- and y-rays can break chemical bonds and produce biologic effects.

energy (in kiloelectron volts\*) and the wavelengths (in angstroms):

$$^{\wedge}A = 12.4/E(\text{keV})$$

For example, x-rays with wavelengths of 0.1 Å correspond to a photon energy of 124 keV

The concept of x-rays being composed of photons is very important in radiobiology. If x-rays are absorbed in living material, energy is deposited in the tissues and cells. This energy is deposited unevenly in discrete packets. The energy in a beam of x-rays is quantized into large individual packets, each of which is big enough to break a chemical bond and initiate the chain of events that culminates in a biologic change. The critical difference between nonionizing and ionizing radiations is the size of the *individual* packets of energy, not the *total* energy involved. A simple calculation illustrates this point. It is shown elsewhere (Chapter 8) that a total-body dose of

about 4 Gy (400 rad) of x-rays given to a human is lethal in many cases. This dose represents an absorption of energy of only about 67 cal, assuming the person to be a "standard man," weighing 70 kg. The smallness of the amount of energy involved can be illustrated in many ways. Converted to heat it would represent a temperature rise of 0.002°C, which would do no harm at all; the same amount of energy in the form of heat is absorbed in drinking one sip of warm coffee. Alternatively, the energy inherent in a lethal dose of x-rays may be compared with mechanical energy or work: It would correspond to the work done in lifting a man about 16 inches from the ground (Fig. 1.4).

Energy in the form of heat or mechanical energy is absorbed uniformly and evenly, and much greater quantities of energy in these forms are required to produce damage in living things. The potency of x-rays, then, is a function not so much of the total energy absorbed as of the size of the individual energy packets. In their biologic effects, electromagnetic radiations are usually considered to be ionizing if they have a photon energy in excess of 124 eV, which corresponds to a wavelength shorter than about  $10^{-6}$  cm.

### Particulate Radiations

Other types of radiation that occur in nature and also are used experimentally are electrons, protons,  $\alpha$ -particles, neutrons, negative  $\tau$ -mesons, and heavy charged ions. Some also are used in radiation therapy and have a potential in diagnostic radiology not yet exploited.

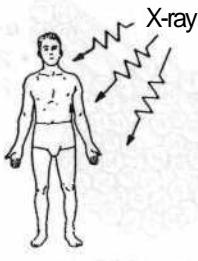
\*The kiloelectron volt (keV) is a unit of energy. It is the energy possessed by an electron that has been accelerated through 1,000 volts. It corresponds to  $1.6 \times 10^{-9}$  ergs.

•The angstrom (Å) is a unit of length equal to  $10^{-8}$  cm.

<sup>†</sup>Quantity of radiation is expressed in roentgens, rads, or gray. The roentgen (R) is the unit of exposure and is related to the ability of x-rays to ionize air. The rad is the unit of absorbed dose and corresponds to an energy absorption of 100 ergs/g. In the case of x- and y-rays an exposure of 1 R results in an absorbed dose in water or soft tissue roughly equal to 1 rad. Officially, the rad has been replaced as a unit by the gray (Gy), which corresponds to an energy absorption of 1 J/kg. Consequently, 1 Gy = 100 rads. Although the gray now commonly is used in Europe, its adoption in everyday practice in the United States has been slow. Often the centigray is used; thus, 1 cGy = 1 rad.

## Total-Body Irradiation

Mass = 70 kg  
 $LD/50/60 = 4 \text{ Gy}$   
 Energy absorbed =



$$70 \times 4 = 280 \text{ joules}$$

$$\frac{280}{4.18} = 67 \text{ calories}$$

## X-ray

**A**

## Drinking Hot Coffee

Excess temperature ( $^{\circ}\text{C}$ ) =  $60^{\circ} - 37^{\circ} = 23^{\circ}$   
 Volume of coffee consumed to equal the energy in the  $LD/50/60$  =  $\frac{23}{23}$

**B**

$$= 3 \text{ mL}$$

$$= 1 \text{ sip}$$

## Mechanical Energy: Lifting a Person

Mass = 70 kg  
 Height lifted to equal the energy in the



$$LD/50/60 = \frac{280}{70 \times 0.0981}$$

$$= 0.4 \text{ m (16 inches)}$$

**C**

**Figure 1.4.** The biologic effect of radiation is determined not by the amount of the energy absorbed but by the photon size, or packet size, of the energy. A: The total amount of energy absorbed in a 70-kg human exposed to a lethal dose of 4 Gy is only 67 cal. B: This is equal to the energy absorbed in drinking one sip of hot coffee. C: It also equals the potential energy imparted by lifting a person about 16 inches.

**Electrons** are small, negatively charged particles that can be accelerated to high energy to a speed close to that of light by means of an electrical device, such as a betatron- or linear accelerator. They are widely used for cancer therapy.

**Protons** are positively charged particles and are relatively massive, having a mass al-

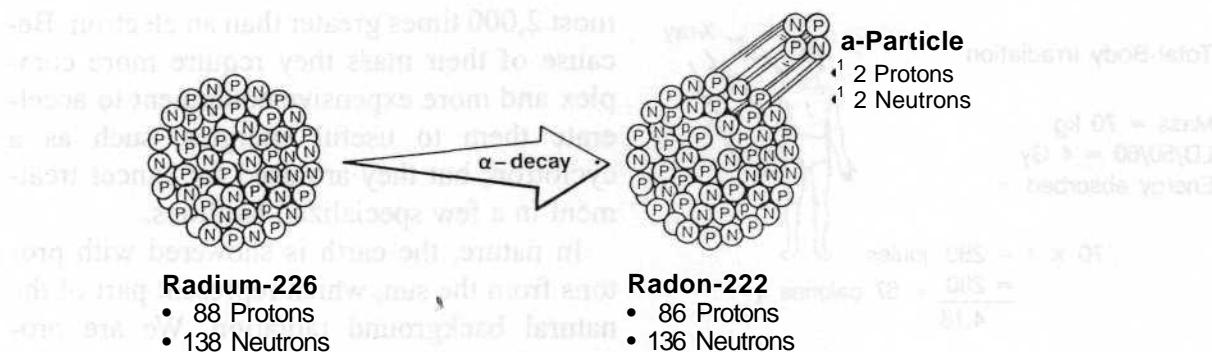
most 2,000 times greater than an electron. Because of their mass they require more complex and more expensive equipment to accelerate them to useful energies, such as a cyclotron, but they are used for cancer treatment in a few specialized facilities.

In nature, the earth is showered with protons from the sun, which represent part of the natural background radiation. We are protected on earth to a large extent by the earth's atmosphere and the magnetic field around the earth, which deflects charged particles away. Protons are a major hazard to astronauts on long-range space missions.

**$\alpha$ -Particles** are nuclei of helium atoms and consist of two protons and two neutrons in close association. They have a net positive charge and therefore can be accelerated in large electrical devices similar to those used for protons.

**$\alpha$ -Particles** also are emitted during the decay of heavy naturally occurring radionuclides, such as uranium and radium (Fig. 1.5).  **$\alpha$ -Particles** are the major source of natural background radiation to the general public. Radon gas seeps out of the soil and builds up inside houses, where together with its decay products it is breathed in and irradiates the lining of the lung. It is estimated that 10,000 to 20,000 cases of lung cancer are caused each year by this means in the United States, mostly in smokers.

**Neutrons** are particles with a mass similar to that of a proton, but they carry no electrical charge. Because they are electrically neutral, they cannot be accelerated in an electrical device. They are produced if a charged particle, such as a deuteron, is accelerated to high energy and then made to impinge on a suitable target material. (A **deuteron** is a nucleus of deuterium and consists of a proton and a neutron in close association.) Neutrons are also emitted as a by-product if heavy radioactive atoms undergo fission, that is, split to form two smaller atoms. Consequently, neutrons are present in large quantities in nuclear reactors and are emitted by some manmade heavy radionuclides. They are also an important component of space radiation and contribute



**Figure 1.5.** Illustration of the decay of a heavy radionuclide by the emission of an  $\alpha$ -particle. An  $\alpha$ -particle is a helium nucleus consisting of two protons and two neutrons. The emission of an  $\alpha$ -particle decreases the atomic number by two and the mass number by four. Note that the radium has changed to another chemical element, radon, as a consequence of the decay.

significantly to the exposure of passengers and crew of high flying jet liners.

**Heavy charged particles** are nuclei of elements such as carbon, neon, argon, or even iron that are positively charged because some or all of the planetary electrons have been stripped from them. To be useful for radiation therapy they must be accelerated to energies of thousands of millions of volts and therefore can be produced in only a few specialized facilities. There is no longer any such facility operational in the United States, but heavy ion therapy is used on a limited scale in Europe and in Japan.

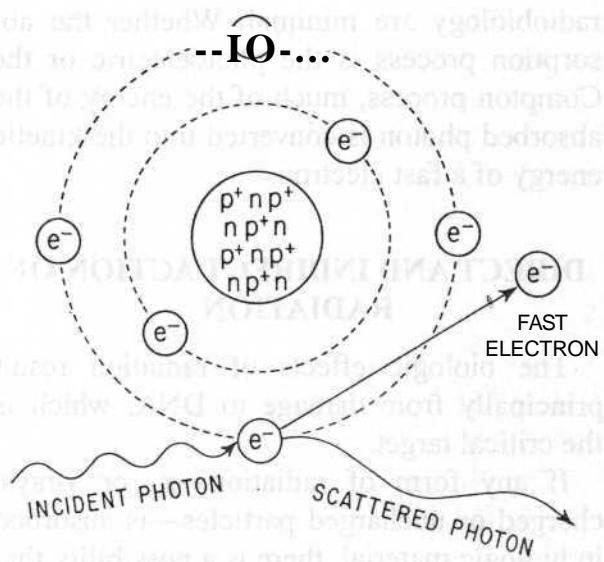
Charged particles of enormous energy are encountered in space and represent a major hazard to astronauts on long missions, such as the proposed trip to Mars. During the lunar missions of the 1970s astronauts "saw" light flashes while their eyes were closed in complete darkness, which turned out to be caused by high-energy iron ions crossing the retina.

### ABSORPTION OF X-RAYS

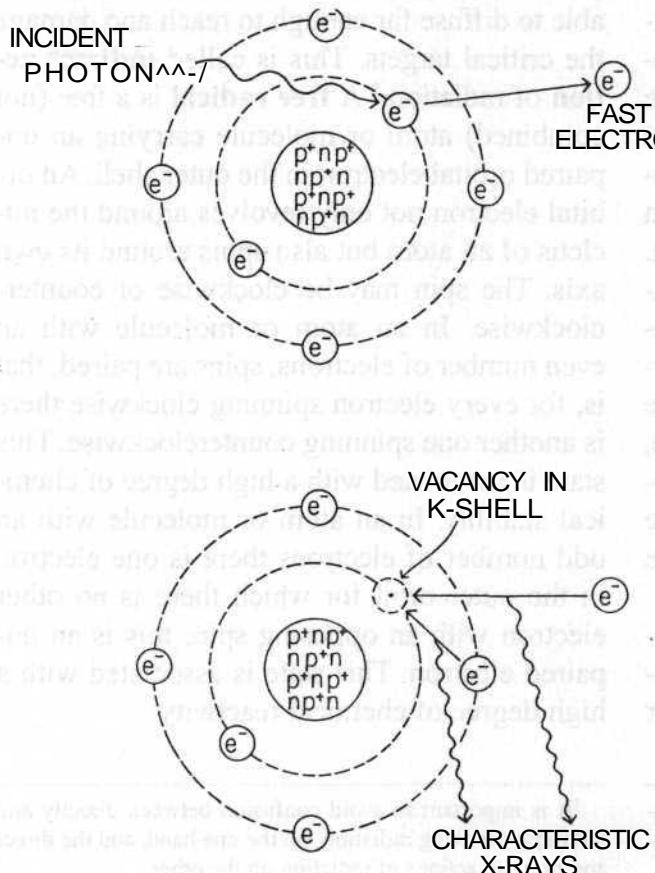
Radiation may be classified as *directly* or *indirectly* ionizing. All of the charged particles previously discussed are **directly ionizing**; that is, provided the individual particles have sufficient kinetic energy, they can disrupt the atomic structure of the absorber through which they pass directly and produce chemical and biologic changes. Electromagnetic radiations (x- and y-rays) are **indirectly**

**ionizing.** They do not produce chemical and biologic damage themselves, but when they are absorbed in the material through which they pass they give up their energy to produce fast-moving charged particles.

The process by which x-ray photons are absorbed depends on the energy of the photons concerned and the chemical composition of the absorbing material. At high energies, characteristic of a cobalt-60 unit or a linear accelerator used for radiotherapy, the **Compton process** dominates. In this process the photon interacts with what is usually referred to as a "free" electron, an electron whose binding energy is negligibly small compared with the photon energy. Part of the energy of the photon is given to the electron as kinetic energy; the photon, with whatever energy remains, continues on its way, deflected from its original path (Fig. 1.6). In place of the incident photon there is a fast electron and a photon of reduced energy, which may go on to take part in further interactions. In any given case the photon may lose a little energy or a lot; in fact, the fraction lost may vary from 0 to 80%. In practice, if an x-ray beam is absorbed by tissue, a vast number of photons interact with a vast number of atoms, and on a statistical basis all possible energy losses occur. The net result is the production of a large number of fast electrons, many of which can ionize other atoms of the absorber, break vital chemical bonds, and initiate the change of events that ultimately is expressed as biologic damage.



**Figure 1.6.** Absorption of an x-ray photon by the Compton process. The photon interacts with a loosely bound planetary electron of an atom of the absorbing material. Part of the photon energy is given to the electron as kinetic energy. The photon, deflected from its original direction, proceeds with reduced energy.



For photon energies characteristic of diagnostic radiology, both Compton and photoelectric absorption processes occur, the former dominating at the higher end of the energy range and the latter being most important at lower energies. In the photoelectric process (Fig. 1.7), the x-ray photon interacts with a bound electron in, for example, the K, L, or M shell of an atom of the absorbing material. The photon gives up all of its energy to the electron; some is used to overcome the binding energy of the electron and release it from its orbit; the remainder is given to the electron as kinetic energy of motion. The kinetic energy (KE) of the ejected electron, therefore, is given by the expression

$$KE = hv - E_B$$

in which  $hv$  is the energy of the incident photon and  $E_B$  is the binding energy of the electron in its orbit. The vacancy left in the atomic shell as a result of the ejection of an electron then must be filled by another electron falling

**Figure 1.7.** Absorption of a photon of x- or y-rays by the photoelectric process. The interaction involves the photon and a tightly bound orbital electron of an atom of the absorber. The photon gives up its energy entirely; the electron is ejected with a kinetic energy equal to the energy of the incident photon less the binding energy that previously held the electron in orbit (**top**). The vacancy is filled either by an electron from an outer orbit or by a free electron from outside the atom (**bottom**). If an electron changes energy levels, the difference in energy is emitted as a photon of characteristic x-rays. For soft tissue these x-rays are of very low energy.

in from an outer shell of the same atom or by a conduction electron from outside the atom. The movement of an electron from one shell to another represents a change of energy states. Because the electron is negatively charged, its movement from a loosely bound to a tightly bound shell represents a decrease of potential energy; this energy change is balanced by the emission of a photon of "characteristic" electromagnetic radiation. In soft tissue, this characteristic radiation has a low energy, typically 0.5 kV, and is of little biologic consequence.

The Compton and photoelectric absorption processes differ in several respects that are vital in the application of x-rays to diagnosis and therapy. The mass absorption coefficient for the Compton process is independent of the atomic number of the absorbing material. By contrast, the mass absorption coefficient for photoelectric absorption varies rapidly with atomic number ( $Z$ )<sup>8</sup> and is, in fact, about proportional to  $Z^3$ .

For diagnostic radiology, photons are used in the energy range in which photoelectric absorption is as important as the Compton process. Because the mass absorption coefficient varies critically with  $Z$ , the x-rays are absorbed to a greater extent by bone because bone contains elements with a high atomic number, such as calcium. This differential absorption in materials of high  $Z$  is one reason for the familiar appearance of the radiograph. On the other hand, for radiotherapy, high-energy photons in the megavoltage range are preferred, because the Compton process is overwhelmingly important. As a consequence, the absorbed dose is about the same in soft tissue, muscle, and bone, so that differential absorption in bone, which posed a problem in the early days in which lower-energy photons were used for therapy, is avoided.

Although the differences among the various absorption processes are of practical importance in radiology, the consequences for

radiobiology are minimal. Whether the absorption process is the photoelectric or the Compton process, much of the energy of the absorbed photon is converted into the kinetic energy of a fast electron.

### DIRECT AND INDIRECT ACTION OF RADIATION

The biologic effects of radiation result principally from damage to DNA, which is the critical target.

If any form of radiation—x- or y-rays, charged or uncharged particles—is absorbed in biologic material, there is a possibility that it will interact directly with the critical targets in the cells. The atoms of the target itself may be ionized or excited, thus initiating the chain of events that leads to a biologic change. This is called **direct action** of radiation (Fig. 1.8); it is the dominant process if radiations with high **linear energy transfer** (LET), such as neutrons or  $\alpha$ -particles, are considered.

Alternatively, the radiation may interact with other atoms or molecules in the cell (particularly water) to produce free radicals that are able to diffuse far enough to reach and damage the critical targets. This is called **indirect action** of radiation.<sup>1</sup> A **free radical** is a free (not combined) atom or molecule carrying an unpaired orbital electron in the outer shell. An orbital electron not only revolves around the nucleus of an atom but also spins around its own axis. The spin may be clockwise or counterclockwise. In an atom or molecule with an even number of electrons, spins are paired; that is, for every electron spinning clockwise there is another one spinning counterclockwise. This state is associated with a high degree of chemical stability. In an atom or molecule with an odd number of electrons there is one electron in the outer orbit for which there is no other electron with an opposing spin; this is an unpaired electron. This state is associated with a high degree of chemical reactivity.

\* $Z$ , the atomic number, is defined as the number of positive charges on the nucleus; it is therefore the number of protons in the nucleus.

<sup>1</sup>It is important to avoid confusion between directly and indirectly ionizing radiation, on the one hand, and the direct and indirect actions of radiation on the other.

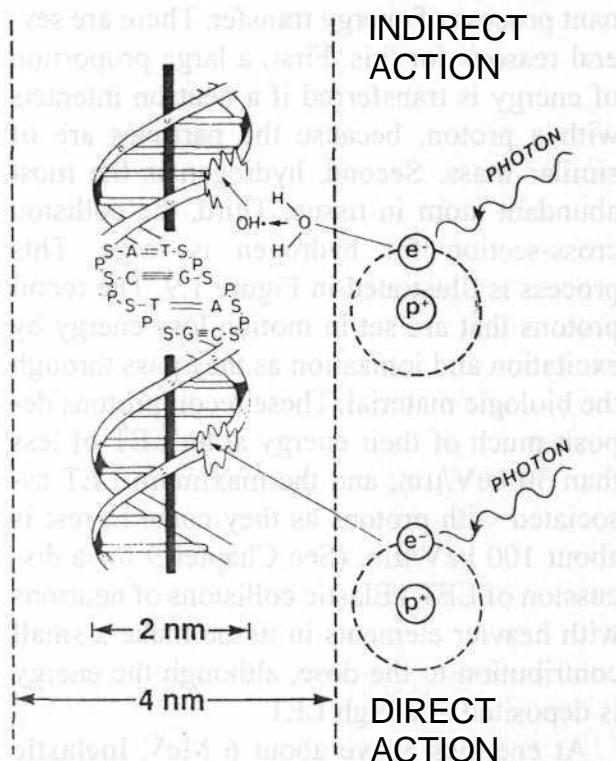
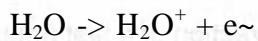


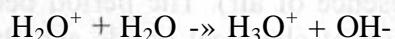
Figure 1.8. Direct and indirect actions of radiation. The structure of DNA is shown schematically. In direct action a secondary electron resulting from absorption of an x-ray photon interacts with the DNA to produce an effect. In indirect action the secondary electron interacts with, for example, a water molecule to produce a hydroxyl radical ( $\text{OH}$ ), which in turn produces the damage to the DNA. The DNA helix has a diameter of about 20 Å (2 nm). It is estimated that free radicals produced in a cylinder with a diameter double that of the DNA helix can affect the DNA. Indirect action is dominant for sparsely ionizing radiation, such as x-rays. S, sugar; P, phosphorus; A, adenine; T, thymine; G, guanine; C, cytosine.

For simplicity, we consider what happens if radiation interacts with a water molecule, because 80% of a cell is composed of water. As a result of the interaction with a photon of x- or y-rays or a charged particle, such as an electron or proton, the water molecule may become ionized. This may be expressed as



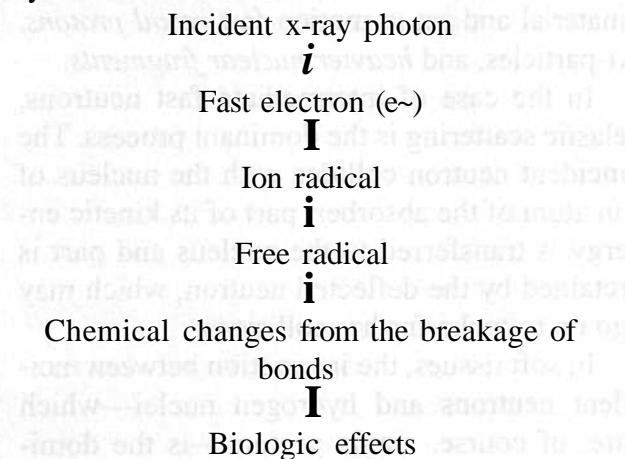
$\text{H}_2\text{O}^+$  is an ion radical. An ion is an atom or molecule that is electrically charged because it has lost an electron. A free radical contains an unpaired electron in the outer shell, as a re-

sult of which it is highly reactive.  $\text{H}_2\text{O}^+$  is charged and has an unpaired electron; consequently, it is both an ion and a free radical. The primary ion radicals have an extremely short lifetime, on the order of  $10^{-10}$  second. They decay to form free radicals, which are not charged but still have an unpaired electron. In the case of water, the ion radical reacts with another water molecule to form the highly reactive hydroxyl radical ( $\text{OH}$ ):



The hydroxyl radical possesses nine electrons; therefore one of them is unpaired. It is a highly reactive free radical and can diffuse a short distance to reach a critical target in a cell. For example, it is thought that free radicals can diffuse to DNA from within a cylinder with a diameter about twice that of the DNA double helix. It is estimated that about two thirds of the x-ray damage to DNA in mammalian cells is caused by the hydroxyl radical. The best evidence for this estimate comes from experiments using free radical scavengers, which can reduce the biologic effect of sparsely ionizing radiations, such as x-rays, by a factor of close to 3. This is discussed further in Chapter 11. Indirect action is illustrated in Figure 1.8. This component of radiation damage can be modified by chemical means—by either protectors or sensitizers—as opposed to the direct action, which cannot be modified.

For the indirect action of x-rays, the chain of events, from the absorption of the incident photon to the final observed biologic change, may be described as follows:



There are vast differences in the time scale involved in these various events. The physics of the process, the initial ionization, may take only  $10^{-15}$  second. The primary radicals produced by the ejection of an electron generally have a lifetime of  $10^{-10}$  second. The OH- radical has a lifetime of about  $10^{-9}$  second in cells, and the DNA radicals formed either by direct ionization or by reaction with OH- radicals have a lifetime of perhaps  $10^{-5}$  second (in the presence of air). The period between the breakage of chemical bonds and the expression of the biologic effect may be hours, days, months, or years, depending on the consequences involved. If cell killing is the result, the biologic effect may be expressed hours to days later, when the damaged cell attempts to divide. If the radiation damage is oncogenic, its expression as an overt cancer may be delayed 40 years. If it is a mutation, in a germ cell leading to heritable changes, it may not be expressed for many generations.

### ABSORPTION OF NEUTRONS

Neutrons are uncharged particles. For this reason they are highly penetrating compared with charged particles of the same mass and energy. They are indirectly ionizing and are absorbed by elastic or inelastic scattering.

Fast neutrons differ basically from x-rays in the mode of their interaction with tissue. *X-ray photons* interact with the *orbital electrons* of atoms of the absorbing material by the Compton or photoelectric process and set in motion fast electrons. *Neutrons*, on the other hand, interact with the nuclei of atoms of the absorbing material and set in motion *fast recoil protons*, *a-particles*, and *heavier nuclear fragments*.

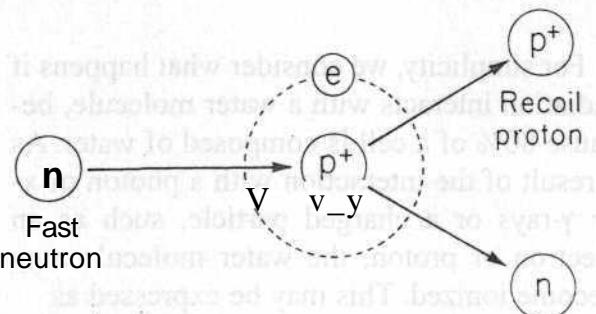
In the case of intermediate fast neutrons, elastic scattering is the dominant process. The incident neutron collides with the nucleus of an atom of the absorber; part of its kinetic energy is transferred to the nucleus and part is retained by the deflected neutron, which may go on to make further collisions.

In soft tissues, the interaction between incident neutrons and hydrogen nuclei—which are, of course, single protons—is the domi-

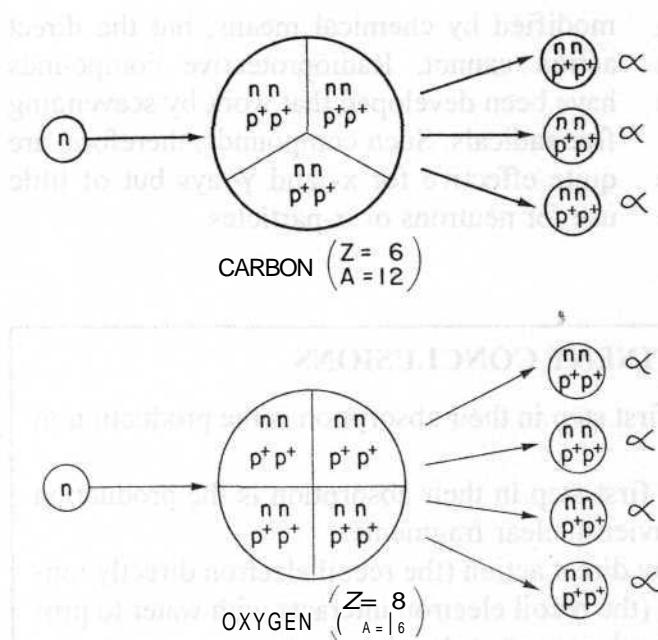
nant process of energy transfer. There are several reasons for this. First, a large proportion of energy is transferred if a neutron interacts with a proton, because the particles are of similar mass. Second, hydrogen is the most abundant atom in tissue. Third, the collision cross-section for hydrogen is large. This process is illustrated in Figure 1.9. The recoil protons that are set in motion lose energy by excitation and ionization as they pass through the biologic material. These recoil protons deposit much of their energy at an LET of less than 30 keV/jim, and the maximum LET associated with protons as they come to rest is about 100 keV/jim. (See Chapter 9 for a discussion of LET.) Elastic collisions of neutrons with heavier elements in tissue make a small contribution to the dose, although the energy is deposited at a high LET.

At energies above about 6 MeV, **inelastic scattering** begins to take place, and it assumes increasing importance as the neutron energy rises.

The neutron may interact with a carbon nucleus to produce three a-particles or with an oxygen nucleus to produce four a-particles (Fig. 1.10). These are known as **spallation** products, which become very important at higher energies. The a-particles produced in this way represent a relatively modest proportion of the total absorbed dose, but they are densely ionizing and have an important effect on the biologic characteristics of the radiation.



**Figure 1.9.** Interaction of a fast neutron with the nucleus of a hydrogen atom of the absorbing material. Part of the energy of the neutron is given to the proton as kinetic energy. The neutron, deflected from its original direction, proceeds with reduced energy.



**Figure 1.10.** The production of spallation products. As the neutron energy rises, the probability increases of a neutron interacting with a carbon or oxygen nucleus to produce three or four  $\alpha$ -particles, respectively. Z, atomic number; A, mass number.

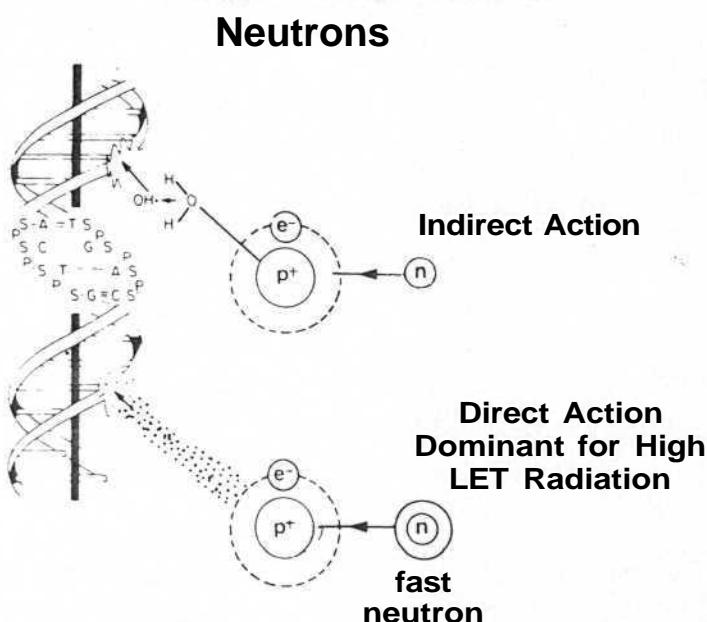
### CONTRAST BETWEEN NEUTRONS AND PHOTONS

X- and  $\gamma$ -rays are indirectly ionizing and give rise to fast-moving secondary electrons. *Fast neutrons* are also indirectly ionizing but

give rise to recoil protons,  $\alpha$ -particles, and heavier nuclear fragments.

The electrons that are set in motion if x-rays are absorbed are very light, negatively charged particles. By contrast, the particles set in motion if neutrons are absorbed are heavy and densely ionizing. They also, for the most part, carry a positive charge, but this difference appears to be relatively trivial biologically. What is important is that they are heavy compared with the electron. A proton, for example, has a mass almost 2,000 times greater than an electron; an  $\alpha$ -particle has a mass four times larger still; and nuclear fragments may occur that are an order of magnitude larger again in mass. The pattern of ionizations and excitations along the tracks of these various charged particles is very different; in particular, the density of ionization is greater for neutrons, pions, and heavy ions than is the case for x- or  $\gamma$ -rays, and this accounts for the dramatic differences in the biologic effects observed. This is discussed further in Chapter 9.

For heavy particles, as for x-rays, the mechanism of biologic effect may be direct or indirect action, but there is a shift in the balance between the two modes of action (Fig. 1.11). For x-rays, indirect action is dominant; for the heavy particles set in motion by neutrons, direct action assumes a greater importance, which increases with the density of ioniza-



**Figure 1.11.** Direct action dominates for more densely ionizing radiations, such as neutrons, because the secondary charged particles produced (protons,  $\alpha$ -particles, and heavier nuclear fragments) result in a dense column of ionizations more likely to interact with the DNA. The local density of DNA radicals produced by direct ionization of DNA is so high that the additional contribution of DNA radicals produced by  $\text{HO}^-$  radical attack does not add substantially to the severity of the lesion.

tion. As the density of ionization increases, the probability of a direct interaction between the particle track and the target molecule (possibly DNA) increases.

It is important to note at this stage that the indirect effect involving free radicals can be

modified by chemical means, but the direct action cannot. Radioprotective compounds have been developed that work by scavenging free radicals. Such compounds, therefore, are quite effective for x- and y-rays but of little use for neutrons or a-particles.

### SUMMARY OF PERTINENT CONCLUSIONS

- X- and y-rays are indirectly ionizing; the first step in their absorption is the production of fast recoil electrons.
- Neutrons are also indirectly ionizing; the first step in their absorption is the production of fast recoil protons, a-particles, and heavier nuclear fragments.
- Biologic effects of x-rays may be caused by direct action (the recoil electron directly ionizes the target molecule) or indirect action (the recoil electron interacts with water to produce an hydroxyl radical, which diffuses to the target molecule).
- About two thirds of the biologic damage by x-rays is caused by indirect action.
- DNA radicals produced by both the direct and indirect action of radiation are modifiable with sensitizers or protectors.
- DNA lesions produced by high-LET radiations involve large numbers of DNA radicals. Chemical sensitizers and protectors are ineffective in modifying such lesions.
- The physics of the absorption process is over in  $10^{-13}$  second; the chemistry takes longer, because the lifetime of the DNA radicals is about  $10^3$  to  $10^5$  second; the biology takes days to months for cell killing, years for carcinogenesis, and generations for heritable damage.

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## DNA Strand Breaks and Chromosomal Aberrations

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### DNA STRAND BREAKS

MEASURING DNA STRAND BREAKS  
CHROMOSOMES AND CELL DIVISION  
THE ROLE OF TELOMERES  
RADIATION-INDUCED CHROMOSOME ABERRATIONS

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### EXAMPLES OF RADIATION-INDUCED

ABERRATIONS  
CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES  
SUMMARY OF PERTINENT CONCLUSIONS

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### DNA STRAND BREAKS

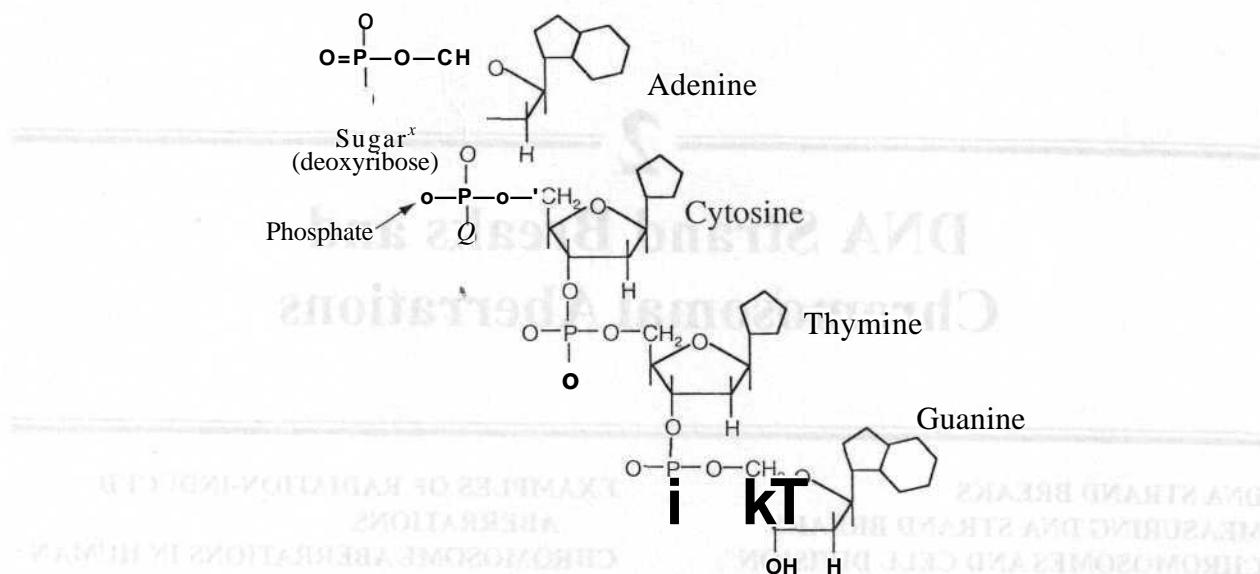
There is strong circumstantial evidence to indicate that DNA is the principal target for the biologic effects of radiation, including cell killing, mutation, and carcinogenesis. A consideration of the biologic effects of radiation therefore must begin logically with a description of the breaks in DNA caused by charged-particle tracks and by the chemical species produced.

Deoxyribonucleic acid (DNA) is a large molecule with a well-known double helix structure. It consists of two strands, held together by hydrogen bonds between the bases. The "backbone" of each strand consists of alternating sugar and phosphate groups. The sugar involved is deoxyribose. Attached to this backbone are four bases, the sequence of which specifies the genetic code. Two of the bases are single-ring groups (pyrimidines); these are thymine and cytosine. Two of the bases are double-ring groups (purines); these are adenine and guanine. The structure of a strand of DNA is illustrated in Figure 2.1. The bases on opposite strands must be complementary; adenine pairs with thymine, guanine

pairs with cytosine. This is illustrated in the simplified model of DNA in Figure 2.2A.

If cells are irradiated with x-rays, many breaks of a single strand occur. These can be observed and scored as a function of dose if the DNA is denatured and the supporting structure stripped away. In intact DNA, however, single-strand breaks are of little biologic consequence as far as a cell killing is concerned because they are repaired readily using the opposite strand as a template (Fig. 2.2B). If the repair is incorrect (misrepair), it may result in a mutation. If both strands of the DNA are broken, and the breaks are well separated (Fig. 2.2C), repair again occurs readily, because the two breaks are handled separately.

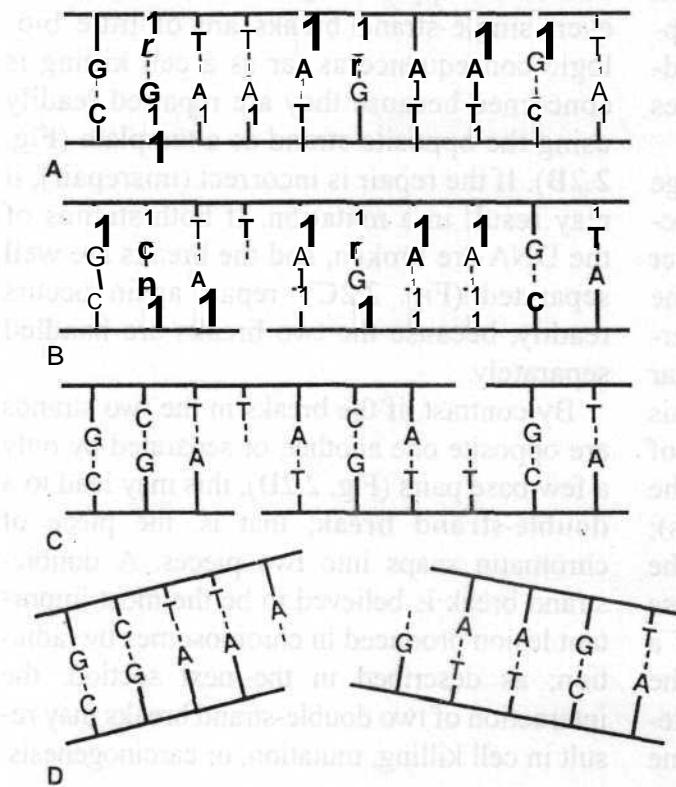
By contrast, if the breaks in the two strands are opposite one another, or separated by only a few base pairs (Fig. 2.2D), this may lead to a **double-strand break**; that is, the piece of chromatin snaps into two pieces. A double-strand break is believed to be the most important lesion produced in chromosomes by radiation; as described in the next section, the interaction of two double-strand breaks may result in cell killing, mutation, or carcinogenesis.



**Figure 2.1.** The structure of a single strand of DNA.

There are many kinds of double-strand breaks, varying in the distance between the breaks on the two DNA strands and the kinds of end groups formed. Their yield in irradiated cells is about 0.04 times that of single-strand breaks, and they are induced linearly with dose, indicating that they are formed by

single tracks of ionizing radiation. Double-strand breaks can be repaired by two basic processes: homologous recombination, requiring an undamaged DNA strand as a participant in the repair, and end-to-end rejoicing via nonhomologous recombination (Fig. 2.3). Homologous recombination, an error-free



**Figure 2.2.** Diagrams of single- and double-strand DNA breaks caused by radiation. A: Two-dimensional representation of the normal DNA helix. The base pairs carrying the genetic code are complementary (i.e., adenine pairs with thymine, guanine pairs with cytosine). B: A break in one strand is of little significance because it is repaired readily, using the opposite strand as a template. C: Breaks in both strands, if well separated, are repaired as independent breaks. D: If breaks occur in both strands and are directly opposite or separated by only a few base pairs, this may lead to a double-strand break in which the chromatin snaps into two pieces. (Courtesy of Dr. John Ward.)

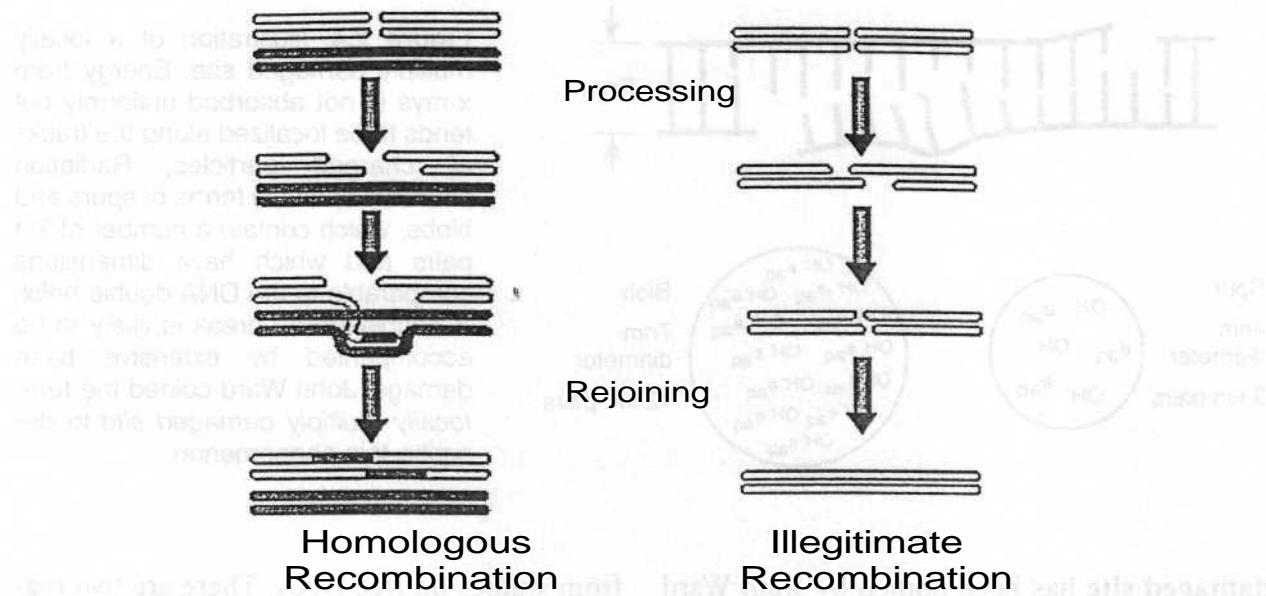
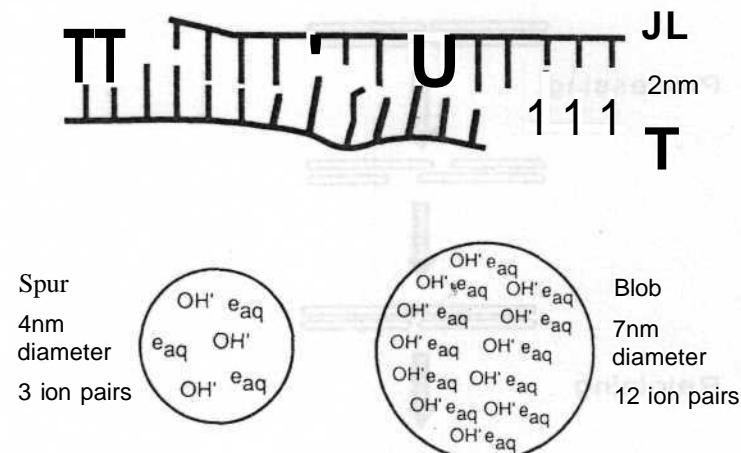


Figure 2.3. Double-strand break repair *via* homologous and nonhomologous (illegitimate) recombination. The lefthand side of the figure shows a double-strand break that has occurred after replication, so that identical sister chromatids are available, in homologous recombination the exposed 3' end invades the homologous duplex, so that the complementary strand acts as a template for gap filling. The breakage of the other strand and subsequent exchanges are not shown. The righthand side of the figures also shows a double-strand break, but in this case no template exists to guide gap filling. Consequently, errors can occur and for this reason it is called illegitimate recombination. (Adapted from Petrini JHJ, Bressan DA, Yao MS: The rad52 epistasis group in mammalian double strand break repair. Semin Immunol 9:181-188, 1997, with permission.)

process, is relatively rare in mammalian cells, and is carried out by proteins similar to the *rad51* gene product of the yeast *S. cerevisiae*. Nonhomologous (illegitimate) recombination is error-prone and probably accounts for many of the premutagenic lesions induced in the DNA of human cells by ionizing radiation. DNA-dependent protein kinase and the Ku proteins participate in this repair process. Recently it has been shown that a protein complex that includes hMre11 and Hrds50 (homologues of proteins involved in the repair of double-strand breaks in *S. cerevisiae*) and p95 (the product of the NBS1 gene) is also involved in the repair of double-strand breaks in human cells.

In practice, the situation is probably much more complicated than illustrated in Figure 2.2D, because both free radicals and direct ionizations may be involved. As described in Chapter 1, the energy from ionizing radiations is not deposited uniformly in the absorbing medium but is located along the tracks of the

charged particles set in motion—electrons in the case of x- or y-rays, protons and oc-particles in the case of neutrons. Radiation chemists speak in terms of "spurs," "blobs," and "short tracks." There is, of course, a full spectrum of energy-event sizes, and it is quite arbitrary to divide them into just three categories, but it turns out to be instructive. A spur contains up to 100 eV of energy and involves, on average, three ion pairs. In the case of x- or y-rays, 95% of the energy deposition events are spurs, which have a diameter of about 4 nm, which is about twice the diameter of the DNA double helix (Fig. 2.4). Blobs are much less frequent for x- or y-rays; they have a diameter of about 7 nm and contain on average about 12 ion pairs (Fig. 2.4). Because spurs and blobs have dimensions similar to the DNA double helix, multiple radical attack occurs if they overlap the DNA helix. There is likely to be a wide variety of complex lesions, including base damage as well as double-strand breaks. The term **locally multiply**



**Figure 2.4.** Illustration of a locally multiply damaged site. Energy from x-rays is not absorbed uniformly but tends to be localized along the tracks of charged particles. Radiation chemists speak in terms of spurs and blobs, which contain a number of ion pairs and which have dimensions comparable to the DNA double helix. A double-strand break is likely to be accompanied by extensive base damage. John Ward coined the term *locally multiply damaged site* to describe this phenomenon.

**damaged site** has been coined by John Ward to describe this phenomenon. Given the size of a spur and the diffusion distance of hydroxyl free radicals, the multiple damage could be spread out up to 20 base pairs. This is illustrated in Figure 2.4, in which a double-strand break is accompanied by base damage and the loss of genetic information.

In the case of densely ionizing radiations, such as neutrons or  $\alpha$ -particles, a greater proportion of blobs is produced. The damage produced, therefore, is qualitatively different to that produced by x- or y-rays and much more difficult for the cell to repair.

### MEASURING DNA STRAND BREAKS

Both single-strand and double-strand DNA breaks can be measured readily by isolating the DNA from irradiated cells and causing the pieces to pass through a porous substrate, such as a gel or a filter. The DNA pieces move under the influence of either flow through the filter or electric field in the gel (using the fact that DNA is positively charged). Smaller pieces move faster and farther than larger pieces of DNA and thus can be separated and counted. The larger the dose of radiation, the more the DNA is broken up. DNA is denatured and lysed by a strong alkaline preparation so that single-strand breaks are measured. Double-strand breaks are measured in a neutral preparation.

DNA in cells is much more resistant to damage by radiation than would be expected

from studies on free DNA. There are two reasons for this: the presence in cells of low molecular weight scavengers that mop up some of the free radicals produced, and the physical protection afforded the DNA by packaging. Certain regions of DNA, particularly actively translating genes, appear to be more sensitive to radiation, and there is some evidence also of sequence-specific sensitivity.

Radiation induces a large number of lesions in DNA, most of which are repaired successfully by the cell. A dose of radiation that induces an average of one lethal event per cell leaves 37% still viable; this is called the  $D_o$  dose and is discussed further in Chapter 3. For mammalian cells,  $D_o$  usually lies between 1 and 2 Gy. The number of DNA lesions per cell detected immediately after such a dose is approximately:

Base damage  $> 1000$

Single-strand breaks about 1000

Double-strand breaks about 40

Cell killing does not correlate at all with single-strand breaks but relates better to double-strand breaks. Agents such as hydrogen peroxide, which produce single-strand breaks efficiently, but very few double-strand breaks, also kill very few cells. On the basis of evidence such as this, it is concluded that double-strand breaks are the most relevant lesions leading to most biologic insults from radiation, including cell killing. The reason for this is that double-strand breaks can lead to chro-

mosomal aberrations, which are discussed in the next section.

## CHROMOSOMES AND CELL DIVISION

The backbone of DNA is made of molecules of sugar and phosphates, which serve as a framework to hold the bases that carry the genetic code. Attached to each sugar molecule is a base: thymine, adenine, guanine, or cytosine. This whole configuration is coiled tightly in a double helix.

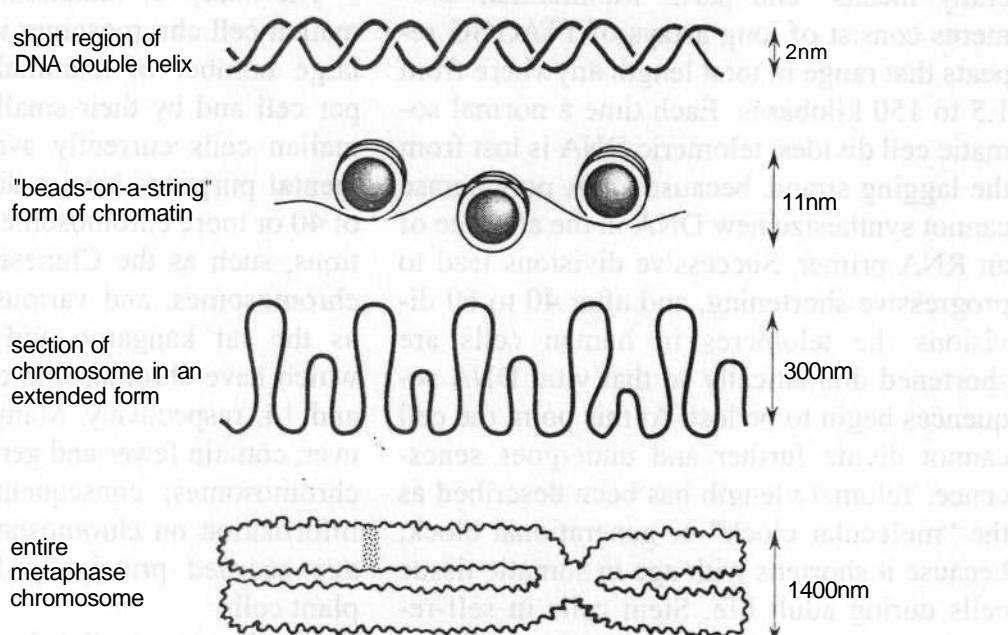
Figure 2.5 is a highly schematized illustration of the way in which an organized folding of the long DNA helix might be achieved as a closely packed series of looped domains wound in a tight helix. The degree of packing also is illustrated by the relative dimensions of the DNA helix and the condensed metaphase chromosome.

The largest part of the life of any somatic cell is spent in interphase, during which the nucleus, in a stained preparation, appears as a lacework of fine, lightly stained material in a translucent, colorless material surrounded by a membrane. In the interphase nucleus in

most cells, one or more bodies of various sizes and shapes, called **nucleoli**, are seen. In most cells, little more than this can be identified with a conventional light microscope. In fact, a great deal is happening during this time: The quantity of DNA in the nucleus doubles as each chromosome lays down an exact replica of itself next to itself. When the chromosomes become visible at mitosis, they are each present in duplicate. Even during interphase, there is good evidence that the chromosomes are not free to move about within the nucleus but are restricted to "domains."

The various events that occur during **mitosis** are reviewed first. The first phase of division is called **prophase**. The beginning of this phase is marked by a thickening of the chromatin and an increase in its stainability as the chromosomes condense into light coils. By the end of prophase each chromosome has a lightly staining constriction known as a **centromere**; extending from the centromere are the arms of the chromosome. Prophase ends when the chromosomes reach maximal condensation and the nuclear membrane disappears, as do any nucleoli.

With the disappearance of the nuclear membrane, the nuclear plasm and the cyto-



**Figure 2.5.** Illustration of the relative sizes of the DNA helix, the various stages of folding and packing of the DNA, and an entire chromosome condensed at metaphase.

plasm mix. Metaphase then follows, in which two events occur simultaneously. The chromosomes move to the center of the cell (*i.e.*, to the cell's equator), and the spindle forms. The spindle is composed of fibers that cross the cell, linking its poles. Once the chromosomes are stabilized at the equator of the cell, their centromeres divide, and metaphase is complete.

The phase that follows, **anaphase**, is characterized by a movement of the chromosomes on the spindle to the poles. Chromosomes appear to be pulled toward the poles of the cell by fibers attached to the centromeres. The arms, particularly the long arms, tend to trail behind.

Anaphase is followed by the last step of the process of mitosis, **telophase**. In this phase the chromosomes, congregated at the poles of the cell, begin to uncoil. The nuclear membrane reappears, as do the nucleoli; and as the phase progresses, the chromosome coils unwind until the nucleus regains the appearance characteristic of interphase.

### THE ROLE OF TELOMERES

**Telomeres** cap and protect the terminal ends of chromosomes. The name *telomere* literally means "end part." Mammalian telomeres consist of long arrays of TTAGGG repeats that range in total length anywhere from 1.5 to 150 kilobases. Each time a normal somatic cell divides, telomeric DNA is lost from the lagging strand, because DNA polymerase cannot synthesize new DNA in the absence of an RNA primer. Successive divisions lead to progressive shortening, and after 40 to 60 divisions the telomeres in human cells are shortened dramatically so that vital DNA sequences begin to be lost. At this point the cell cannot divide further and undergoes senescence. Telomere length has been described as the "molecular clock" or generational clock, because it shortens with age in somatic tissue cells during adult life. Stem cells in self-renewing tissues, and cancer cells in particular, avoid this problem of aging by activating the enzyme telomerase. Telomerase is a reverse

transcriptase that includes the complementary sequence to the TTAGGG repeats and so continually rebuilds the chromosome ends to offset the degradation that occurs with each division. In this way the cell becomes immortal.

In tissue culture, immortalization of cells, that is, cells that pass through a "crisis" and continue to be able to divide beyond the normal limit, is associated with telomere stabilization and activity of telomerase.

Virtually all human tumor-cell lines and approximately 90% of human cancer biopsy specimens exhibit telomerase activity. By contrast, normal human somatic tissues, other than stem cells, do not possess detectable levels of this enzyme. It is an attractive hypothesis that both immortalization and carcinogenesis are associated with telomerase expression.

### RADIATION-INDUCED CHROMOSOME ABERRATIONS

In the traditional study of chromosome aberrations, the effects of ionizing radiations are described in terms of their appearance when a preparation is made at the first metaphase after exposure to radiation. This is the time at which the structure of the chromosomes can be discerned.

The study of radiation damage in mammalian cell chromosomes is hampered by the large number of mammalian chromosomes per cell and by their small size. Most mammalian cells currently available for experimental purposes have a diploid complement of 40 or more chromosomes. There are exceptions, such as the Chinese hamster, with 22 chromosomes, and various marsupials, such as the rat kangaroo and woolly opossum, which have chromosome complements of 12 and 14, respectively. Many plant cells, however, contain fewer and generally much larger chromosomes; consequently, until recently, information on chromosomal radiation damage accrued principally from studies with plant cells.

If cells are irradiated with x-rays, breaks are produced in the chromosomes. The broken ends appear to be "sticky" and can rejoin

with any other sticky end. It would appear, however, that a broken end cannot join with a normal, unbroken chromosome, although this is controversial. Once breaks are produced, different fragments may behave in a variety of ways:

1. The breaks may restitute, that is, rejoin in their original configuration. In this case, of course, nothing amiss is visible at the next mitosis.
2. The breaks may fail to rejoin and give rise to an aberration, which is scored as a deletion at the next mitosis.
3. Broken ends may reassort and rejoin other broken ends to give rise to chromosomes that appear to be grossly distorted if viewed at the following mitosis.

This is an oversimplified account; whether actual breaks occur in the chromosomes at the time of irradiation is not known, nor is the biologic significance of "stickiness" understood.

The aberrations seen at metaphase are of two classes: *chromosome* aberrations and *chromatid* aberrations. **Chromosome aberrations** result if a cell is irradiated early in interphase, before the chromosome material has been duplicated. In this case the radiation-induced break is in a single strand of chromatin; during the DNA synthetic phase that follows, this strand of chromatin lays down an identical strand next to itself and replicates the break that has been produced by the radiation. This leads to a chromosome aberration visible at the next mitosis, because there is an identical break in the corresponding points of a pair of chromatin strands. If, on the other hand, the dose of radiation is given later in interphase, after the DNA material has doubled and the chromosomes consist of two strands of chromatin, then the aberrations produced are called **chromatid aberrations**. In regions removed from the centromere, chromatid arms may be fairly well separated, and it is reasonable to suppose that the radiation might break one chromatid without breaking its sister chromatid, or at least not in the same place. A break that occurs in a single chromatid arm after chromosome replication and leaves the

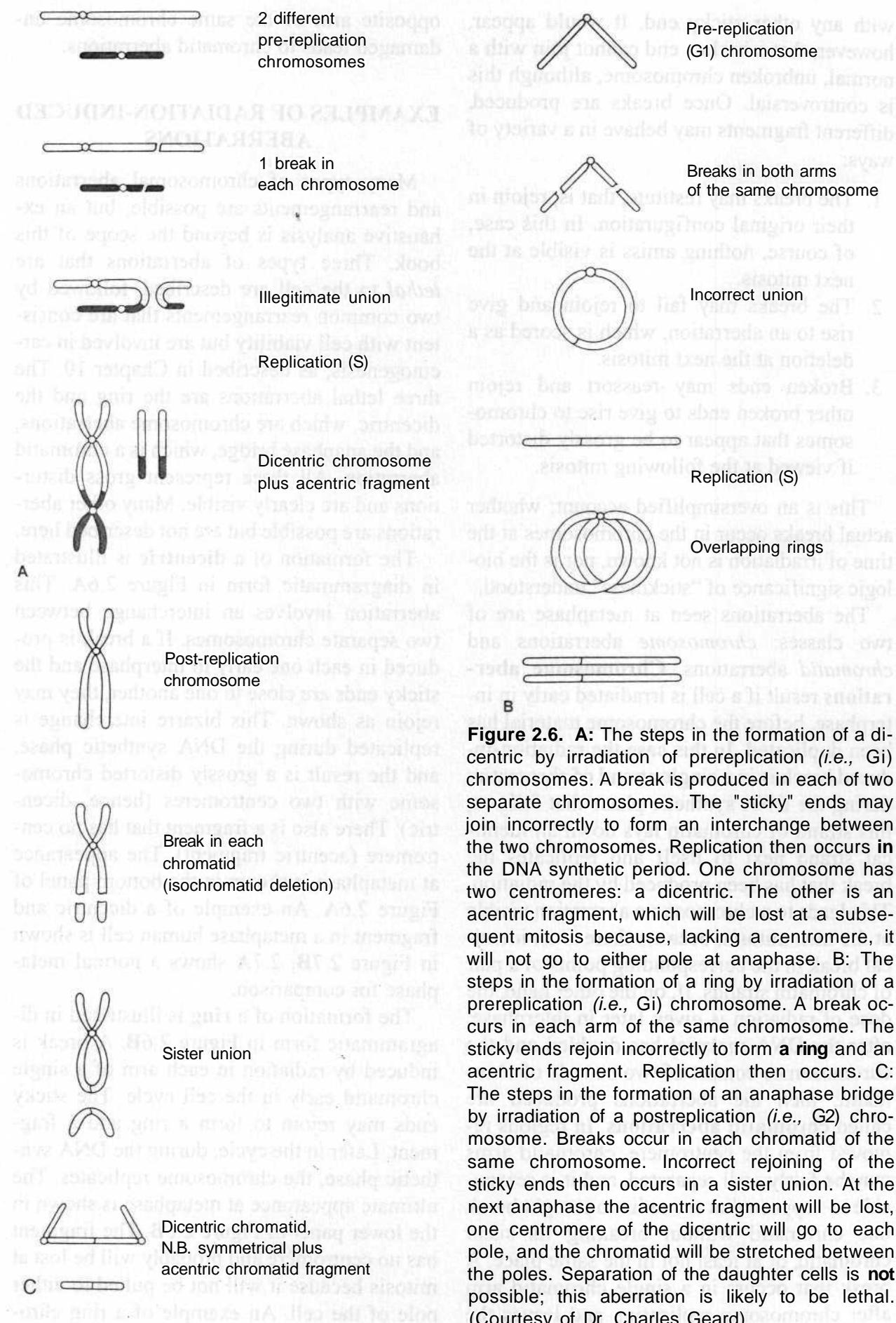
opposite arm of the same chromosome undamaged leads to chromatid aberrations.

### EXAMPLES OF RADIATION-INDUCED ABERRATIONS

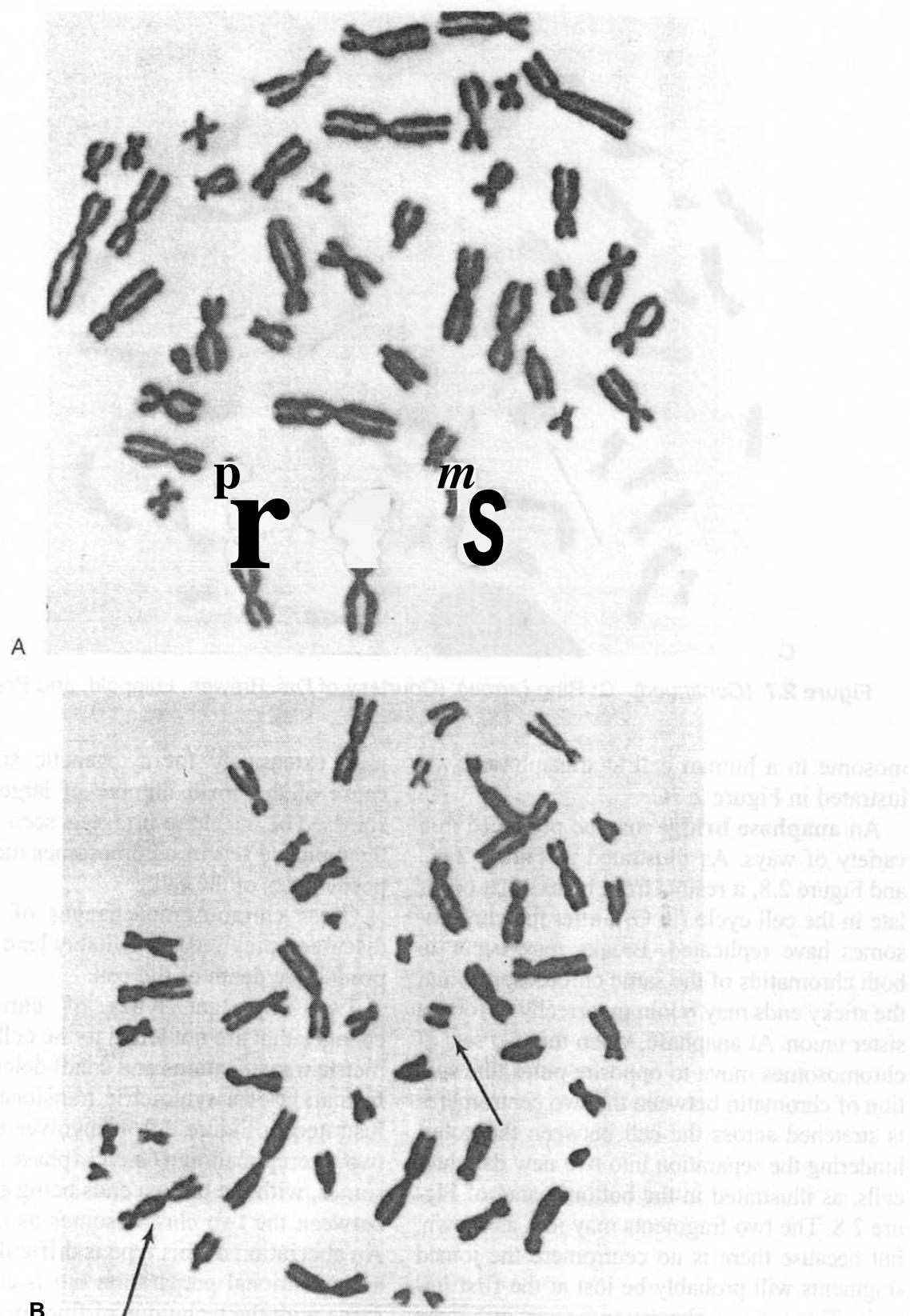
Many types of chromosomal aberrations and rearrangements are possible, but an exhaustive analysis is beyond the scope of this book. Three types of aberrations that are *lethal* to the cell are described, followed by two common rearrangements that are consistent with cell viability but are involved in carcinogenesis, as described in Chapter 10. The three lethal aberrations are the ring and the dicentric, which are chromosome aberrations, and the anaphase bridge, which is a chromatid aberration. All three represent gross distortions and are clearly visible. Many other aberrations are possible but are not described here.

The formation of a dicentric is illustrated in diagrammatic form in Figure 2.6A. This aberration involves an interchange between two separate chromosomes. If a break is produced in each one early in interphase and the sticky ends are close to one another, they may rejoin as shown. This bizarre interchange is replicated during the DNA synthetic phase, and the result is a grossly distorted chromosome with two centromeres (hence, dicentric). There also is a fragment that has no centromere (acentric fragment). The appearance at metaphase is shown in the bottom panel of Figure 2.6A. An example of a dicentric and fragment in a metaphase human cell is shown in Figure 2.7B; 2.7A shows a normal metaphase for comparison.

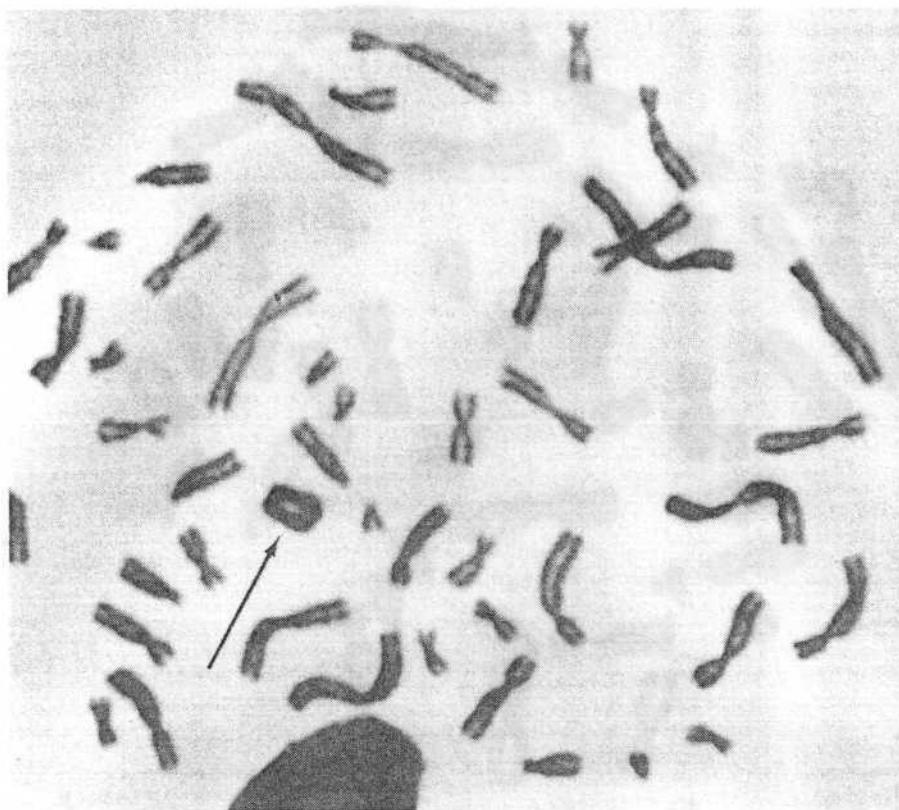
The formation of a ring is illustrated in diagrammatic form in Figure 2.6B. A break is induced by radiation in each arm of a single chromatid early in the cell cycle. The sticky ends may rejoin to form a ring and a fragment. Later in the cycle, during the DNA synthetic phase, the chromosome replicates. The ultimate appearance at metaphase is shown in the lower panel of Figure 2.6B. The fragment has no centromere and probably **will** be lost at mitosis because it will not be pulled to either pole of the cell. An example of a ring chro-



**Figure 2.6. A:** The steps in the formation of a dicentric by irradiation of prereplication (*i.e.*, G<sub>1</sub>) chromosomes. A break is produced in each of two separate chromosomes. The "sticky" ends may join incorrectly to form an interchange between the two chromosomes. Replication then occurs **in** the DNA synthetic period. One chromosome has two centromeres: a dicentric. The other is an acentric fragment, which will be lost at a subsequent mitosis because, lacking a centromere, it will not go to either pole at anaphase. **B:** The steps in the formation of a ring by irradiation of a prereplication (*i.e.*, G<sub>1</sub>) chromosome. A break occurs in each arm of the same chromosome. The sticky ends rejoin incorrectly to form **a ring** and an acentric fragment. Replication then occurs. **C:** The steps in the formation of an anaphase bridge by irradiation of a postreplication (*i.e.*, G<sub>2</sub>) chromosome. Breaks occur in each chromatid of the same chromosome. Incorrect rejoining of the sticky ends then occurs in a sister union. At the next anaphase the acentric fragment will be lost, one centromere of the dicentric will go to each pole, and the chromatid will be stretched between the poles. Separation of the daughter cells is **not** possible; this aberration is likely to be lethal. (Courtesy of Dr. Charles Geard)



**Figure 2.7.** Radiation-induced chromosome aberrations in human leukocytes viewed at metaphase. A: Normal metaphase. B: Dicentric and fragment (arrows). (Continued).



**Figure 2.7 (Continued). C: Ring (arrow).** (Courtesy of Drs. Brewen, Luippold, and Preston.)

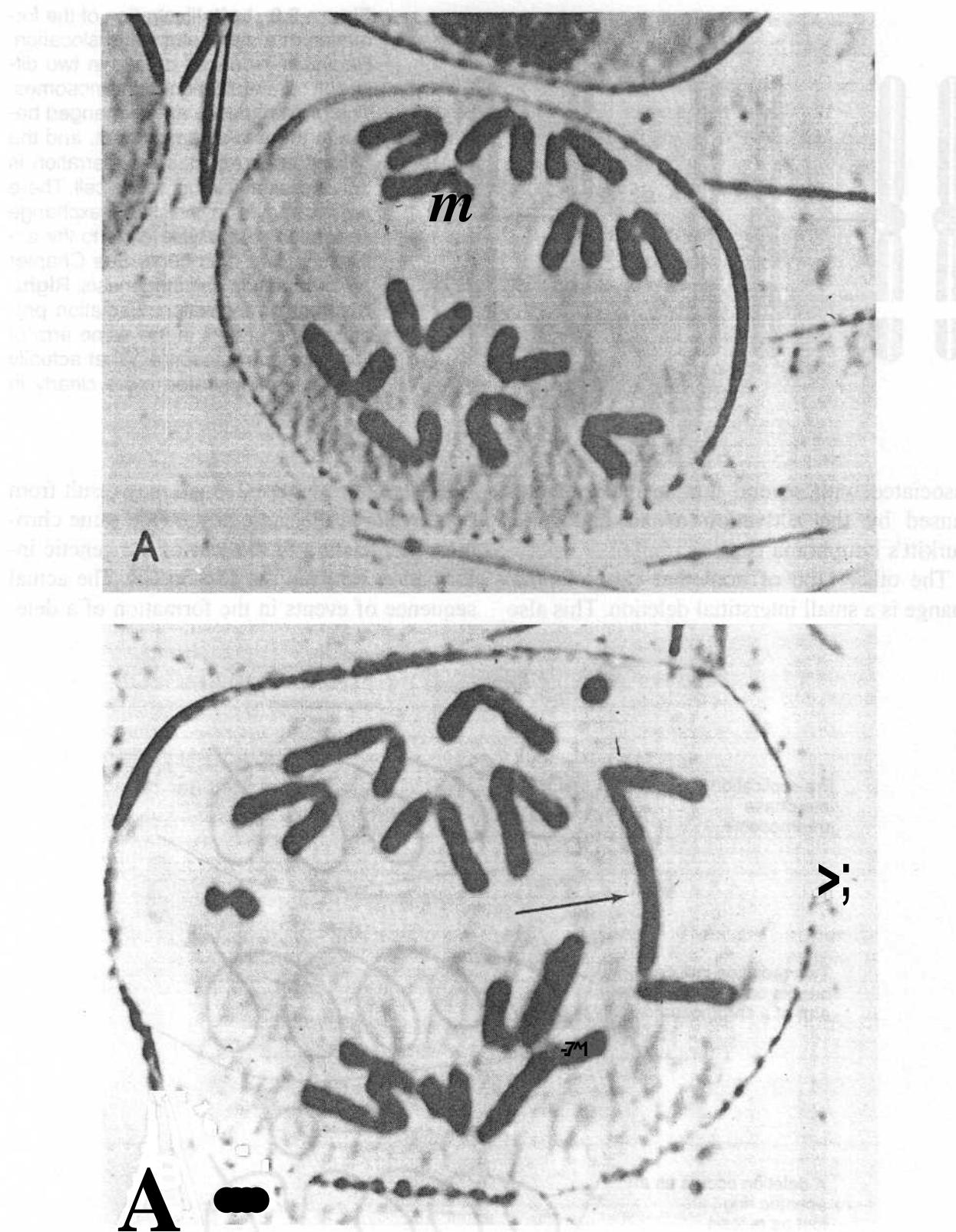
mosome in a human cell at metaphase is illustrated in Figure 2.7C.

An **anaphase bridge** may be produced in a variety of ways. As illustrated in Figure 2.6C and Figure 2.8, it results from breaks that occur late in the cell cycle (in G<sub>2</sub>), after the chromosomes have replicated. Breaks may occur in both chromatids of the same chromosome, and the sticky ends may rejoin incorrectly to form a sister union. At anaphase, when the two sets of chromosomes move to opposite poles, the section of chromatin between the two centromeres is stretched across the cell between the poles, hindering the separation into two new daughter cells, as illustrated in the bottom panel of Figure 2.8. The two fragments may join as shown, but because there is no centromere the joined fragments will probably be lost at the first mitosis. This type of aberration occurs in human cells and is essentially always lethal. It is hard to demonstrate, because preparations of human chromosomes usually are made by accumulating cells at metaphase but the bridge is only evident at anaphase. Figure 2.8 is an anaphase preparation of *Tmescantia paludosa*, a plant

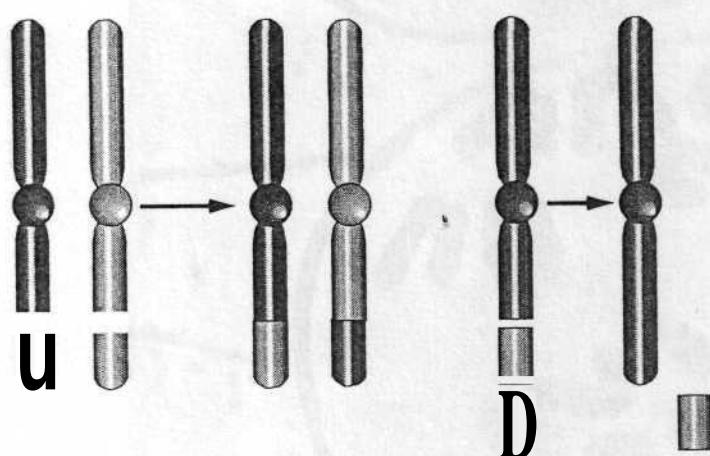
used extensively for cytogenetic studies because of the small number of large chromosomes. The anaphase bridge is seen clearly as the replicate sets of chromosomes move to opposite poles of the cell.

Gross chromosome changes of the types discussed previously inevitably lead to the reproductive death of the cell.

Two important types of chromosomal changes that are not lethal to the cell are symmetric translocations and small deletions. The formation of a symmetric translocation is illustrated in Figure 2.9. It involves a break in two prereplication (*i.e.*, G<sub>1</sub>-phase) chromosomes, with the broken ends being exchanged between the two chromosomes as illustrated. An aberration of this type is difficult to see in a conventional preparation but is easy to observe with the technique of fluorescent *in situ* hybridization, or *chromosome painting*, as it commonly is called. Probes are available for every human chromosome that make them fluorescent in a bright color. Exchange of material between two different chromosomes then is readily observable. A translocation is



**Figure 2.8.** Anaphase chromosome preparation of *Tradescantia pallida*. A: Normal anaphase. B: Bridge and fragment resulting from radiation (arrow). (Courtesy of Drs. Brewen, Luippold, and Preston.)

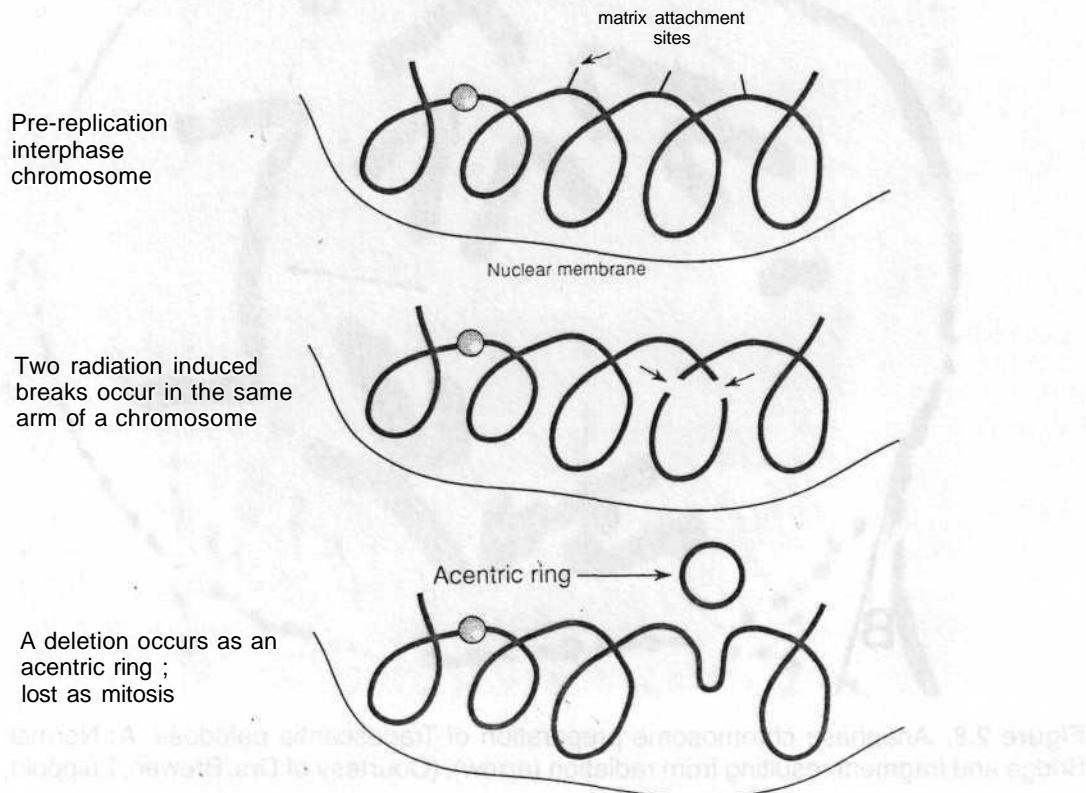


**Figure 2.9.** **Left:** illustration of the formation of a symmetrical translocation. Radiation produces breaks in two different prereplication chromosomes. The broken pieces are exchanged between the two chromosomes, and the "sticky" ends rejoin. This aberration is not necessarily lethal to the cell. There are examples in which an exchange aberration of this type leads to the activation of an oncogene. See Chapter 19 on radiation carcinogenesis. **Right:** Diagram of a deletion. Radiation produces two breaks in the same arm of the same chromosome. What actually happens is illustrated more clearly in Figure 2.10.

**associated** with several human malignancies caused by the activation of an oncogene; Burkitt's lymphoma is an example.

The other type of nonlethal chromosomal change is a small interstitial deletion. This also

is illustrated in Figure 2.9 and may result from two breaks in the same arm of the same chromosome, leading to the loss of the genetic information between the two breaks. The actual sequence of events in the formation of a dele-



**Figure 2.10.** Illustration of the formation of a deletion by ionizing radiation in an interphase chromosome. It is easy to imagine how two breaks may occur (by a single or two different charged particles) in such a way as to isolate a loop of DNA. The "sticky" ends rejoin, and the deletion is lost at a subsequent mitosis because it has no centromere. This loss of DNA may include the loss of a suppressor gene and lead to a malignant change. See Chapter 19 on radiation carcinogenesis.

tion is easier to understand from Figure 2.10, which shows an interphase chromosome. It is a simple matter to imagine how two breaks may isolate a loop of DNA—an acentric ring—which is lost at a subsequent mitosis. A deletion may be associated with carcinogenesis if the lost genetic material includes a suppressor gene. This is discussed further in Chapter 11 on radiation carcinogenesis.

The interaction between breaks in different chromosomes is by no means random. There is great heterogeneity in the sites at which deletions and exchanges between different chromosomes occur; for example, chromosome 8 is particularly sensitive to exchanges. As mentioned previously, each chromosome is restricted to a domain, and most interactions occur at the edges of domains, which probably involves the nuclear matrix. Active chromosomes are therefore those with the biggest surface area to their domains.

### CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES

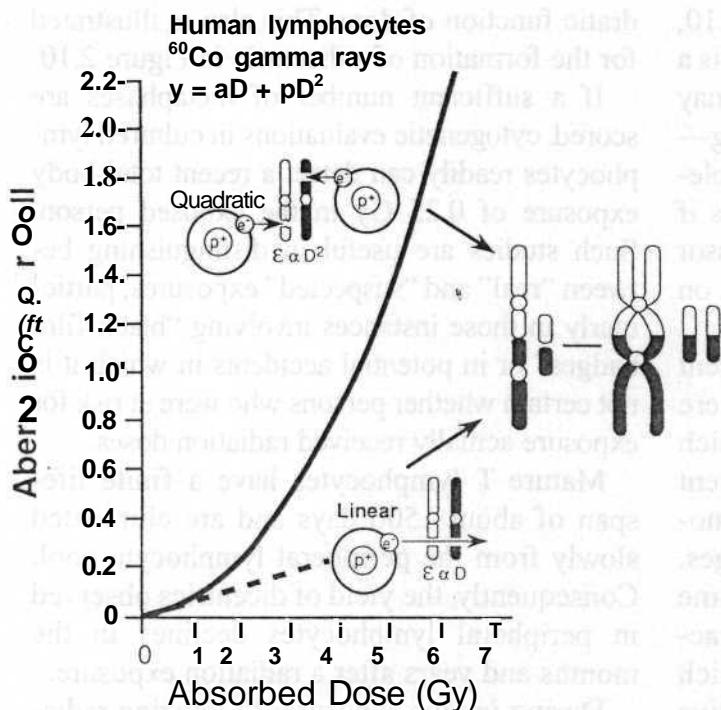
Chromosomal aberrations in peripheral lymphocytes have been used widely as biomarkers of radiation exposure. In blood samples obtained for cytogenetic evaluation within a few days to a few weeks after total-body irradiation, the frequency of asymmetric aberrations in the lymphocytes (dicentrics and rings) reflects the dose received. Lymphocytes in the blood sample are stimulated to divide with a mitogen such as phytohemagglutinin and are arrested at metaphase, and the incidence of rings and dicentrics is scored. The dose can be estimated by comparison with *in vitro* cultures exposed to known doses. Figure 2.11 shows a dose-response curve for aberrations in human lymphocytes produced by  $\gamma$ -rays. The data are fitted by a linear-quadratic relationship, as would be expected, because rings and dicentrics result from the interaction of two chromosome breaks, as previously described. The linear component is a consequence of the two breaks resulting from a single charged particle. If the two breaks result from different charged particles, the probability of an interaction is a qua-

dritic function of dose. This also is illustrated for the formation of a dicentric in Figure 2.10.

If a sufficient number of metaphases are scored, cytogenetic evaluations in cultured lymphocytes readily can detect a recent total-body exposure of 0.25 Gy in the exposed person. Such studies are useful in distinguishing between "real" and "suspected" exposures, particularly in those instances involving "black film badges" or in potential accidents in which it is not certain whether persons who were at risk for exposure actually received radiation doses.

Mature T lymphocytes have a finite lifespan of about 1500 days and are eliminated slowly from the peripheral lymphocyte pool. Consequently, the yield of dicentrics observed in peripheral lymphocytes declines in the months and years after a radiation exposure.

During *in vivo* exposures to ionizing radiation, chromosome aberrations are induced not only in mature lymphocytes but also in lymphocyte progenitors in marrow, nodes, or other organs. The stem cells that sustain asymmetric aberrations (such as dicentrics) die in attempting a subsequent mitosis, but those that sustain a symmetric nonlethal aberration (such as a translocation) survive and pass on the aberration to their progeny. Consequently, dicentrics are referred to as "unstable" aberrations, because their number declines with time after irradiation. Symmetric translocations, by contrast, are referred to as "stable" aberrations, because they persist for many years. Either type of aberration can be used to estimate dose soon after irradiation, but if many years have elapsed, scoring dicentrics underestimates the dose, and only stable aberrations such as translocations give accurate pictures. Until recently, translocations were much more difficult to observe than dicentrics, but now the technique of fluorescent *in situ* hybridization makes the scoring of such symmetric aberrations a relatively simple matter. The frequency of translocations assessed in this way correlates with total-body dose in exposed persons even after more than 50 years, as was shown in a recent study of the survivors of the atomic-bomb attacks on Hiroshima and Nagasaki.



**Figure 2.11.** The frequency of chromosomal aberrations (dicentrics and rings) is a linear-quadratic function of dose because the aberrations are the consequence of the interaction of two separate breaks. At low doses, both breaks may be caused by the same electron; the probability of an exchange aberration is proportional to dose ( $D$ ). At higher doses, the two breaks are more likely to be caused by separate electrons. The probability of an exchange aberration is proportional to the square of the dose ( $D^2$ ).

### SUMMARY OF PERTINENT CONCLUSIONS

- Many single-strand breaks are produced in DNA by radiation but are repaired readily using the opposite DNA strand as a template.
- Breaks in both strands, if well separated, also are repaired readily, because they are handled individually.
- Breaks in both strands that are opposite, or separated by only a few base pairs, may lead to a double-strand break.
- Energy from x-rays is deposited unevenly in "spurs" and "blobs." This may lead to *multiply damaged sites*, that is, a combination of a double-strand break and base damage.
- There is good reason to believe that double-strand breaks rather than single-strand breaks lead to important biologic endpoints, including cell death.
- Radiation-induced breakage and incorrect rejoining in prereplication chromosomes (G<sub>1</sub> phase) may lead to *chromosome aberrations*.
- Radiation-induced breakage and incorrect rejoining in postreplication chromosomes (S or G<sub>2</sub> phase) may lead to *chromatid aberrations*.
- Principal aberrations include *dicentrics*, *rings*, *acentric fragments*, *translocations*, and *anaphase bridges*.
- There is a good correlation between cells killed and cells with asymmetric exchange aberrations (i.e., dicentrics or rings).
- The incidence of most radiation-induced aberrations is a linear-quadratic function of dose.
- Scoring aberrations in lymphocytes from peripheral blood may be used to estimate total-body doses in humans accidentally irradiated. The lowest single dose that can be detected readily is 0.25 Gy (25 rad).
- Dicentrics are "unstable" aberrations; they are lethal to the cell and are not passed on to progeny. Consequently, the incidence of dicentrics declines slowly with time after exposure.
- Translocations are "stable" aberrations; they persist for many years because they are not lethal to the cell and are passed on to the progeny.

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# 3

## Cell Survival Curves

### REPRODUCTIVE INTEGRITY

### THE *IN VITRO* SURVIVAL CURVE

### THE SHAPE OF THE SURVIVAL CURVE

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### MAMMALIAN CELLS IN CULTURE

### SURVIVAL CURVE SHAPE AND

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### CALCULATIONS OF TUMOR CELL KILL

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### MICROORGANISMS

### SUMMARY OF PERTINENT CONCLUSIONS

## REPRODUCTIVE INTEGRITY

A **cell-survival curve** describes the relationship between the radiation dose and the proportion of cells that survive. What is meant by "survival?" Cell survival, or its converse, cell death, may mean different things in different contexts; therefore, a precise definition is essential. For differentiated cells that do not proliferate, such as nerve, muscle, or secretory cells, death can be defined as the loss of a specific function. For proliferating cells, such as stem cells in the hematopoietic system or the intestinal epithelium, loss of the capacity for sustained proliferation—that is, loss of *reproductive integrity*—is an appropriate definition. This is sometimes called **reproductive death**. This is certainly the endpoint measured with cells cultured *in vitro*.

This definition reflects a narrow view of radiobiology. A cell still may be physically present and apparently intact, may be able to make proteins or synthesize DNA, and may even be able to struggle through one or two mitoses; but if it has lost the capacity to divide

indefinitely and produce a large number of progeny, it is by definition dead; it has not survived. A survivor that has retained its reproductive integrity and is able to proliferate indefinitely to produce a large clone or colony is said to be *clonogenic*.

This definition is generally relevant to the radiobiology of whole animals and plants and their tissues. It has particular relevance to the radiotherapy of tumors. For a tumor to be eradicated, it is only necessary that cells be "killed" in the sense that they are rendered unable to divide and cause further growth and spread of the malignancy. Cells may die by different mechanisms, as is described here subsequently. For most cells, death while attempting to divide, that is, mitotic death, is the dominant mechanism following irradiation. For some cells, programmed cell death or apoptosis is important. Whatever the mechanism, the outcome is the same: The cell loses its ability to proliferate indefinitely, that is, its reproductive integrity.

In general, a dose of 100 Gy (10,000 rad) is necessary to destroy cell function in nonpro-

liferating systems. By contrast, the mean lethal dose for loss of proliferative capacity is usually less than 2 Gy (200 rad).

### THE IN VITRO SURVIVAL CURVE

The capability of a single cell to grow into a large colony, which can be seen easily with the naked eye, is a convenient proof that it has retained its reproductive integrity. The loss of this ability as a function of radiation dose is described by the dose-survival curve.

With modern techniques of tissue culture it is possible to take a specimen from a tumor or from many normal regenerative tissues, chop it into small pieces, and prepare a single-cell suspension by the use of the enzyme trypsin, which dissolves and loosens the cell membrane. If these cells are seeded into a culture dish, covered with an appropriate complex growth medium, and maintained at 37°C under aseptic conditions, they attach to the surface, grow, and divide.

In practice, most fresh explants grow well for a few weeks but subsequently peter out and die. A few pass through a crisis and continue to

grow for many years. Every few days the culture must be "farmed": the cells are removed from the surface with trypsin, most of the cells are discarded, and the culture flask is reseeded with a small number of cells, which quickly repopulate the culture flask. These are called **established cell lines**; they have been used extensively in experimental cellular radiobiology.

Survival curves are so basic to an understanding of much of radiobiology that it is worthwhile to go through the steps involved in a typical experiment using an established cell line in culture.

Cells from an actively growing stock culture are prepared into a suspension by the use of trypsin, which causes the cells to round up and detach from the surface of the culture vessel. The number of cells per unit volume of this suspension is counted in a hemocytometer or with an electronic counter. In this way, for example, 100 individual cells may be seeded into a dish; if this dish is incubated for 1 to 2 weeks, each single cell divides many times and forms a colony that is easily visible with the naked eye, especially if it is fixed and stained (Fig. 3.1). All cells making up each colony are the progeny of

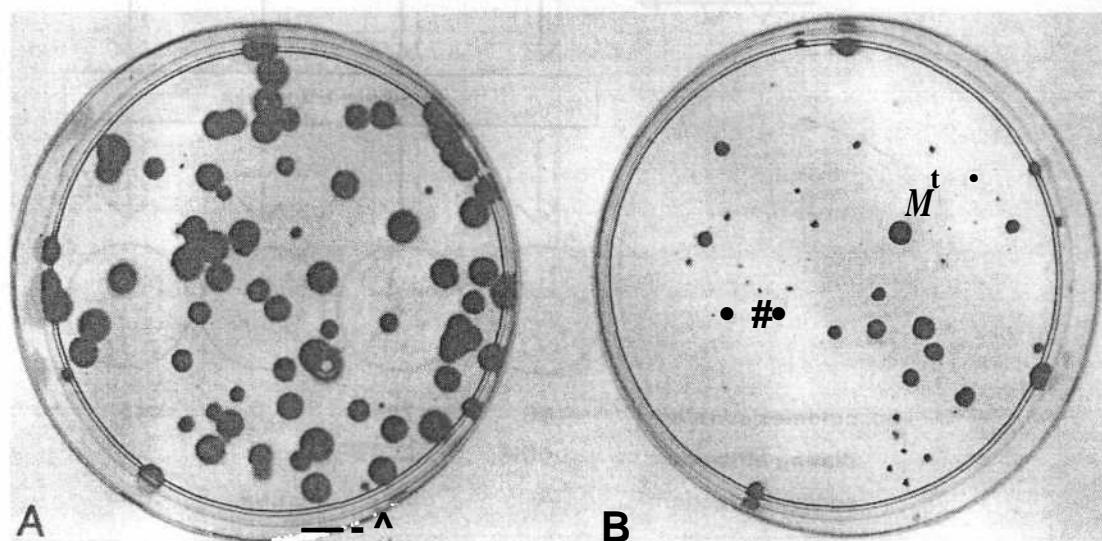


Figure 3.1. Colonies obtained with Chinese hamster cells cultured in vitro. A: In this unirradiated control dish 100 cells were seeded and allowed to grow for 7 days before being stained. There are 70 colonies; therefore the plating efficiency is 70/100, or 70%. B: Two thousand cells were seeded and then exposed to 800 rad (8 Gy) of x-rays. There are 32 colonies on the dish. Thus:

$$\text{Surviving fraction} = \frac{\text{Colonies counted}}{\text{Colonies seeded}} \times \frac{1}{\text{PE}/100}$$

$$= \frac{32}{2000} \times .7$$

$$= 0.023$$

a single ancestor. For a nominal 100 cells seeded into the dish, the number of colonies counted may be expected to be in the range of 50 to 90. Ideally, it should be 100, but it seldom is for a variety of reasons, including suboptimal growth medium, errors and uncertainties in

counting the cell suspension, and the trauma of trypsinization and handling. The term **plating efficiency** indicates the percentage of cells seeded that grow into colonies. There are 70 colonies on the control dish in Figure 3.1 A; therefore the plating efficiency is 70%.

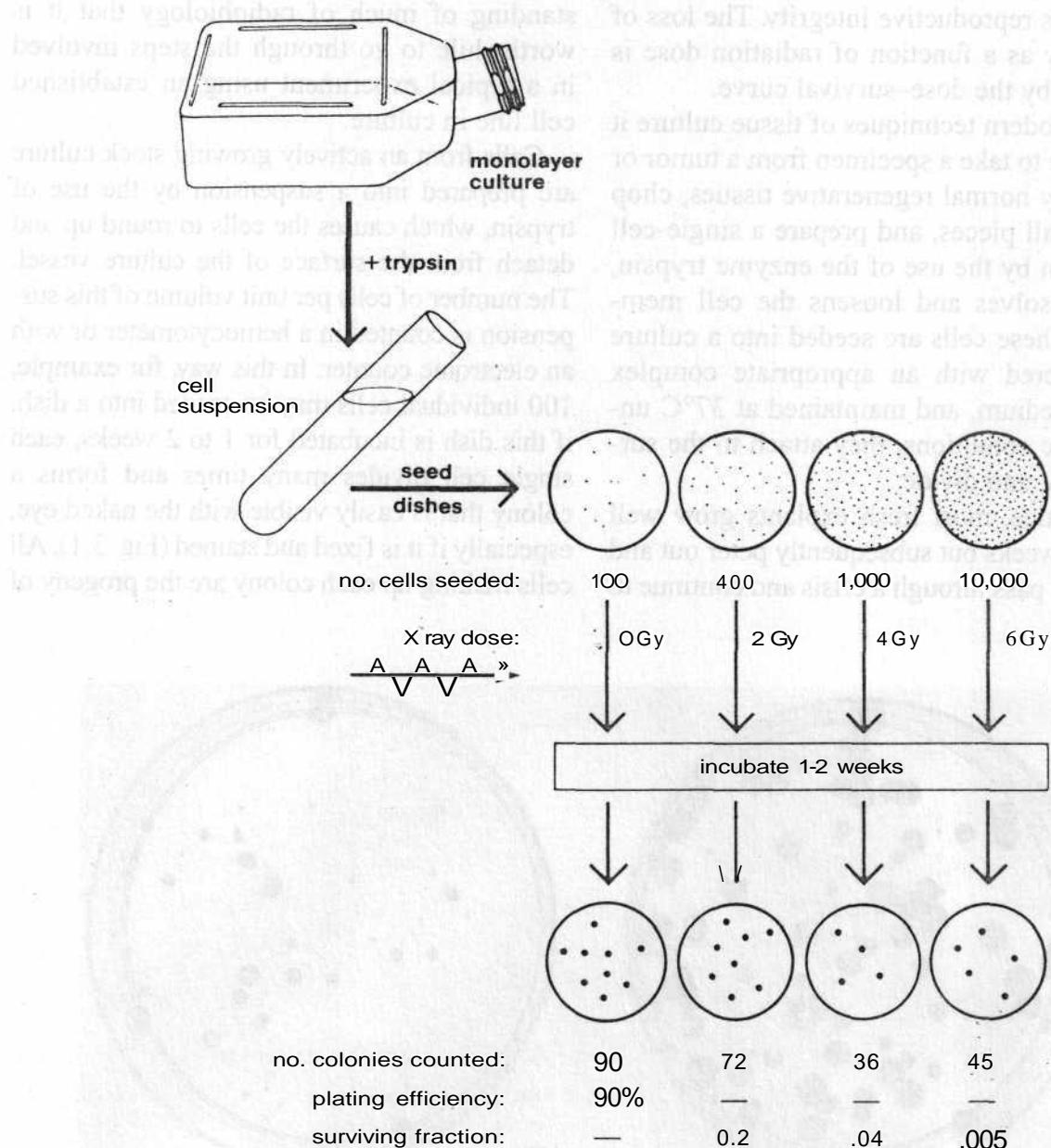


Figure 3.2. The cell culture technique used to generate a cell-survival curve. Cells from a stock culture are prepared into a single-cell suspension by trypsinization, and the cell concentration is counted. Known numbers of cells are inoculated into petri dishes and irradiated. They then are allowed to grow until the surviving cells produce macroscopic colonies that can be counted readily. The number of cells per dish initially inoculated varies with the dose, so that the number of colonies surviving is in the range that can be counted conveniently. Surviving fraction is the ratio of colonies produced to cells plated, with a correction necessary for plating efficiency (*i.e.*, for the fact that not all cells plated grow into colonies, even in the absence of radiation).

If a parallel dish is seeded with cells, exposed to a dose of 8 Gy (800 rad) of x-rays, and incubated for 1 to 2 weeks before being fixed and stained, then the following may be observed: (1) Some of the seeded single cells are still single and have not divided, and in some instances the cells show evidence of nuclear deterioration as they die an apoptotic death; (2) some cells have managed to complete one or two divisions to form a tiny abortive colony; and (3) some cells have grown into large colonies that differ little from the unirradiated controls, although they may vary more in size. These cells are said to have survived, because they have retained their reproductive integrity.

In the example shown in Figure 3.IB, 2,000 cells had been seeded into the dish exposed to 8 Gy (800 rad). Because the plating efficiency is 70%, 1,400 of the 2,000 cells plated would have grown into colonies if the dish had not been irradiated. In fact, there are only 32 colonies on the dish in Figure 3.IB; the fraction of cells surviving the dose of x-rays is thus

$$\frac{32}{1400} = 0.023$$

In general, the surviving fraction is given by

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

This process is repeated so that estimates of survival are obtained for a range of doses. The number of cells seeded per dish is adjusted so that a countable number of colonies results: Too few reduces statistical significance; too many cannot be counted accurately, because they tend to merge into one another. The technique is illustrated in Figure 3.2. This technique, and the survival curve that results, does not distinguish the mode of cell death, that is, whether the cells died mitotic or apoptotic deaths.

### THE SHAPE OF THE SURVIVAL CURVE

Survival curves for mammalian cells usually are presented in the form shown in Figure

3.3, with dose plotted on a linear scale and surviving fraction on a logarithmic scale. Qualitatively, the shape of the survival curve can be described in relatively simple terms. At "low doses" for sparsely ionizing (low linear energy transfer) radiations, such as x-rays, the survival curve starts out straight on the log-linear plot with a finite initial slope; that is, the surviving fraction is an exponential function of dose. At higher doses, the curve bends. This bending or curving region extends over a dose range of a few grays (a few hundred rads). At very high doses the survival curve often tends to straighten again; the surviving fraction returns to being an exponential function of dose. In general, this does not occur until doses in excess of those used as daily fractions in radiotherapy have been reached.

By contrast, for densely ionizing (high linear energy transfer) radiations, such as **Ot**-particles or low-energy neutrons, the cell-survival curve is a straight line from the origin; that is, survival approximates to an exponential function of dose (Fig. 3.3).

Although it is a simple matter to qualitatively describe the shape of the cell-survival curve, finding an explanation of the biologic observations in terms of biophysical events is another matter. Many biophysical models and theories have been proposed to account for the shape of the mammalian cell-survival curve. Almost all can be used to deduce a curve shape that is consistent with experimental data, but it is never possible to choose among different models or theories on the basis of goodness of fit to experimental data. The biologic data are not sufficiently precise, nor are the predictive theoretic curves sufficiently different, for this to be possible.

Two descriptions of the shape of survival curves are discussed briefly, with a minimum of mathematics (Fig. 3.3). First, the multitarget model that was widely used for many years still has some merit. In this model, the survival curve is described in terms of an **initial slope**,  $D_i$ , resulting from single-event killing; a **final slope**,  $D_o$ , resulting from multiple-event killing; and some quantity (either  $n$  or  $D_q$ ) to represent the size or width of the shoulder of the curve.

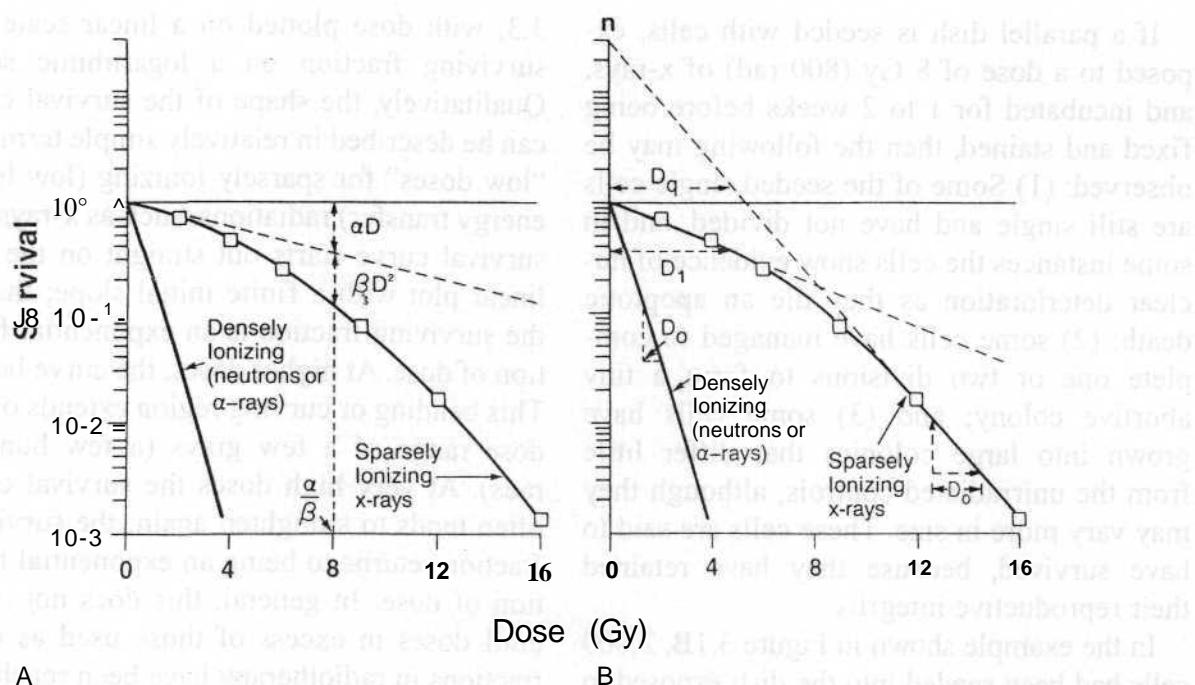


Figure 3.3. Shape of survival curve for mammalian cells exposed to radiation. The fraction of cells surviving is plotted on a logarithmic scale against dose on a linear scale. For  $\alpha$ -particles or low-energy neutrons (said to be densely ionizing) the dose-response curve is a straight line from the origin (*i.e.*, survival is an exponential function of dose). The survival curve can be described by just one parameter, the slope. For x- or y-rays (said to be sparsely ionizing), the dose-response curve has an initial linear slope, followed by a shoulder; at higher doses the curve tends to become straight again. A: The experimental data are fitted to a linear-quadratic function. There are two components of cell killing: one is proportional to dose ( $\alpha D$ ), the other is proportional to the square of the dose ( $(\beta D^2)$ ). The dose at which the linear and quadratic components are equal is the ratio  $\alpha/(\beta)$ . The linear-quadratic curve bends continuously but is a good fit to experimental data for the first few decades of survival. B: The curve is described by the initial slope ( $D_i$ ), the final slope ( $D_o$ ), and a parameter that represents the width of the shoulder, either  $n$  or  $D_q$ .

The quantities  $D_i$  and  $D_o$  are the reciprocals of the initial and final slopes. In each case it is the dose required to reduce the fraction of surviving cells to 37% of its previous value. As illustrated in Figure 3.3B,  $D_i$ , the initial slope, is the dose required to reduce the fraction of surviving cells to 0.37 on the initial straight portion of the survival curve. The final slope,  $D_o$ , is the dose required to reduce survival from 0.1 to 0.37, or from 0.01 to 0.0037, and so on. Because the surviving fraction is on a logarithmic scale and the survival curve becomes straight at higher doses, the dose required to reduce the cell population by a given factor (to 0.37) is the same at all survival levels. It is, on average, the dose required to deliver one inactivating event per cell.

The *extrapolation number*,  $n$ , is a measure of the width of the shoulder. If  $n$  is large (*eg*,

10 or 12), the survival curve has a broad shoulder. If  $n$  is small (*eg*, 1.5 to 2), the shoulder of the curve is narrow. Another measure of shoulder width is the *quasithreshold* dose, shown as  $D_q$  in Figure 3.3. This sounds like a term invented by a committee, which in a sense it is. An easy way to remember its meaning is to think of the hunchback of Notre Dame. When the priest was handed the badly deformed infant who was to grow up to be the hunchback, he cradled him in his arms and said, "We will call him Quasimodo—he is almost a person!" Similarly, the quasithreshold dose is almost a threshold dose. It is defined as the dose at which the straight portion of the survival curve, extrapolated backward, cuts the dose axis drawn through a survival fraction of unity. A threshold dose is the dose be-

low which there is no effect. There is no dose below which radiation produces no effect, so there can be no true threshold dose;  $D_q$ , the quasithreshold dose, is the closest thing.

At first sight this might appear to be an awkward parameter, but in practice it has certain merits that becomes apparent in subsequent discussion. The three parameters,  $\alpha$ ,  $D_0$ , and  $D_q$  are related by the expression

$$\log(1 - S) = D_q/D_0$$

The linear-quadratic model has taken over as the model of choice to describe survival curves. It is a direct development of the relation used to describe exchange-type chromosome aberrations that are clearly the result of an interaction between two separate breaks. This is discussed in some detail in Chapter 2.

The linear-quadratic model assumes that there are two components to cell killing by radiation, one that is proportional to dose and one that is proportional to the square of the dose. The notion of a component of cell inactivation that varies with the square of the dose introduces the concept of dual-radiation action. This idea goes back to the early work with chromosomes in which many chromosome aberrations are clearly the result of two separate breaks. (Examples discussed in Chapter 2 are rings, dicentrics, and anaphase bridges, all of which are likely to be lethal to the cell.)

By this model the expression for the cell-survival curve is

$$S = e^{-\alpha D - \beta D^2}$$

in which  $S$  is the fraction of cells surviving a dose  $D$ , and  $\alpha$  and  $\beta$  are constants. The components of cell killing that are proportional to dose and to the square of the dose are equal if

$$\alpha D = [3\beta D^2]$$

or

$$D = \alpha/\beta$$

This is an important point that bears repeating: The linear and quadratic contributions to cell killing are equal at a dose that is equal to the ratio of  $\alpha$  to  $\beta$ . This is illustrated in Figure 3.3A.

A characteristic of the linear-quadratic formulation is that the resultant cell-survival curve is continuously bending; there is no final straight portion. This does not coincide with what is observed experimentally if survival curves are determined down to seven or more decades of cell killing, in which case the dose-response relationship closely approximates to a straight line in a log-linear plot; that is, cell killing is an exponential function of dose. In the first decade or so of cell killing and up to any doses used as daily fractions in clinical radiotherapy, however, the linear-quadratic model is an adequate representation of the data. It has the distinct advantage of having only two adjustable parameters,  $\alpha$  and  $\beta$ .

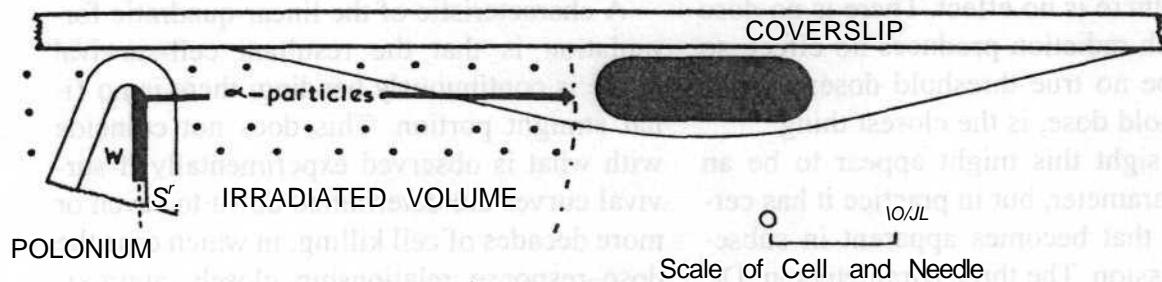
## MECHANISMS OF CELL KILLING

### DNA as the Target

Abundant evidence shows that the sensitive sites for radiation-induced cell lethality are located in the nucleus as opposed to the cytoplasm.

Early experiments with nonmammalian systems, such as frog's eggs, amoebae, and algae, were designed so that either the cell nucleus or the cytoplasm could be irradiated selectively with a microbeam. The results indicated that the nucleus was very much more radiosensitive than the cytoplasm.

In later experiments, Munro irradiated mammalian cells with short-range  $\alpha$ -particles from polonium (Fig. 3.4). Individual cells attached to a glass coverslip were studied. A large proportion of the cytoplasm could be irradiated by locating a polonium microneedle at a known distance from the cell; the  $\alpha$ -particles from the polonium have a definite well-defined range, and it is possible to ensure that the  $\alpha$ -particles irradiate the cytoplasm without reaching the nucleus. By the same token, it is possible to position the  $\alpha$ -source above the cell and to irradiate the nucleus with  $\alpha$ -particles but irradiate a minimal amount of cytoplasm. It was found that large numbers of  $\alpha$ -particles, corresponding to a dose in excess of 250 Gy (25,000 rad), if delivered to the cy-



**Figure 3.4.** Irradiation of part of the cytoplasm of a cultured Chinese hamster cell by  $\alpha$ -particles from a polonium-tipped microneedle. The irradiated volume is limited by the range of the particles. (From Munro TR: Radiat Res 42:451, 1970).

toplasm, had no effect on cell proliferation. By contrast, the penetration of a few  $\alpha$ -particles a distance of 1 or 2  $\mu\text{m}$  into the nucleus could prove to be lethal.

**Evidence for chromosomal DNA as the principal target for cell killing is circumstantial but overwhelming.** There is evidence that the nuclear membrane may be involved, also; indeed, the one does not exclude the other, because some portion of the DNA may be intimately involved with the membrane during some portions of the cell cycle.

The evidence implicating the chromosomes, specifically the DNA, as the primary target for radiation-induced lethality may be summarized as follows:

1. Cells are killed by radioactive tritiated thymidine incorporated into the DNA. The radiation dose results from short-range  $\alpha$ -particles and is therefore very localized.
2. Certain structural analogues of thymidine, particularly the halogenated pyrimidines, are incorporated selectively into DNA in place of thymidine if substituted in cell-culture growth medium. This substitution dramatically increases the radiosensitivity of the mammalian cells to a degree that increases as a function of the amount of the incorporation. Substituted deoxyuridines, which are not incorporated into DNA, have no such effect on cellular radiosensitivity.
3. Factors that modify cell lethality, such as variation in the type of radiation, oxygen concentration, and dose rate, also affect

the production of chromosome damage in a fashion qualitatively and quantitatively similar. This is at least *prima facie* evidence to indicate that damage to the chromosomes is implicated in cell lethality.

4. Early work showed a relationship between virus size and radiosensitivity; later work showed a better correlation with nucleic-acid volume. The radiosensitivity of a wide range of plants has been correlated with the mean interphase chromosome volume, which is defined as the ratio of nuclear volume to chromosome number. The larger the mean chromosome volume, the greater the radiosensitivity.
5. A direct correlation has been observed in hamster cells between aberrant chromosomes at the first postirradiation division and the failure of the cell to form a colony. Also, a correlation has been observed between chromosome fragments in irradiated plants and the failure of the pollen grain to germinate.

This accumulated evidence strongly indicates that **chromosomal DNA is the principal target for radiation-induced lethality.**

#### Apoptotic and Mitotic Death

Apoptosis was first described by Kerr and colleagues as a particular set of changes at the microscopic level associated with cell death. The word *apoptosis* is derived from the Greek word meaning "falling off," as in petals from flowers, or leaves from trees. Apoptosis, or

programmed cell death, is common in embryonic development in which some tissues become obsolete. It is the mechanism, for example, by which tadpoles lose their tails.

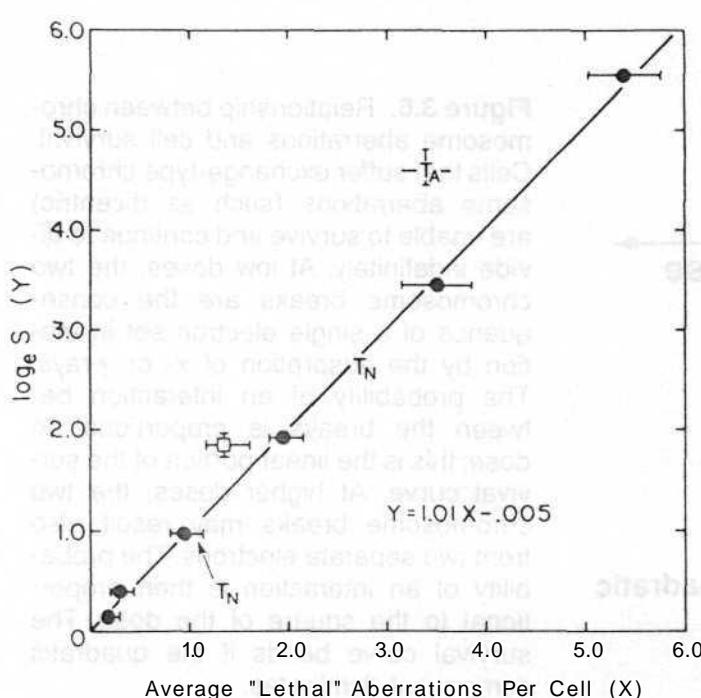
This form of cell death is characterized by a stereotyped sequence of morphologic events. One of the earliest steps a cell takes if it is committed to die in a tissue is to cease communicating with its neighbors. This is evident as the dying cell rounds up and detaches from its neighbors. Condensation of the chromatin at the nuclear membrane and fragmentation of the nucleus are then evident. The cell shrinks because of cytoplasmic condensation, resulting from cross-linking of proteins and loss of water. Eventually the cell separates into a number of membrane-bound fragments of differing sizes termed *apoptotic bodies*, which may contain cytoplasm only, or nuclear fragments. The morphologic hallmark of apoptosis is the condensation of the nuclear chromatin in either crescents around the periphery of the nucleus, or a group of spheric fragments.

Double-strand breaks occur in the linker regions between nucleosomes, to produce DNA fragments that are multiples of approximately 185 base pairs. These fragments result in the

characteristic ladders seen in gels. In contrast, necrosis causes a diffuse "smear" of DNA in gels. Apoptosis occurs in normal tissues, as described previously, and also can be induced in some normal tissues and in some tumors by radiation.

As a mode of radiation-induced cell death, apoptosis is highly cell-type dependent. Hemopoietic and lymphoid cells are particularly prone to rapid radiation-induced cell death by the apoptotic pathway. In most tumor cells, mitotic cell death is at least as important as apoptosis, and in some cases it is the only mode of cell death. A number of genes appear to be involved. First, apoptosis after radiation seems commonly to be a p53-dependent process; bcl-2 is a suppressor of apoptosis.

The most common form of cell death from radiation is mitotic death: Cells die in attempting to divide because of damaged chromosomes. Death may occur in the first or a late division following irradiation. Many authors have reported a close quantitative relationship between cell killing and the induction of specific chromosomal aberrations. The results of one of the most elegant studies, by Cornforth and Bedford, are shown in Figure 3.5. It should be noted



**Figure 3.5.** Relationship between the average number of "lethal" aberrations per cell (i.e., asymmetric exchange-type aberrations such as dicentrics and rings) and the log of the surviving fraction in AC 1522 normal human fibroblasts exposed to x-rays. There is virtually a one-to-one correlation. (From Cornforth MN, Bedford JS: A quantitative comparison of potentially lethal damage repair and the rejoicing of interphase chromosome breaks in low passage normal human fibroblasts. Radiat Res 111:385-405, 1987, with permission.)

that these experiments were carried out in a cell line where apoptosis is not observed. The log of the surviving fraction is plotted against the average number of putative "lethal" aberrations per cell, that is, asymmetric exchange-type aberrations such as rings and dicentrics. There is virtually a one-to-one correlation. In addition, there is an excellent correlation between the fraction of cells surviving and the fraction of cells without visible aberrations.

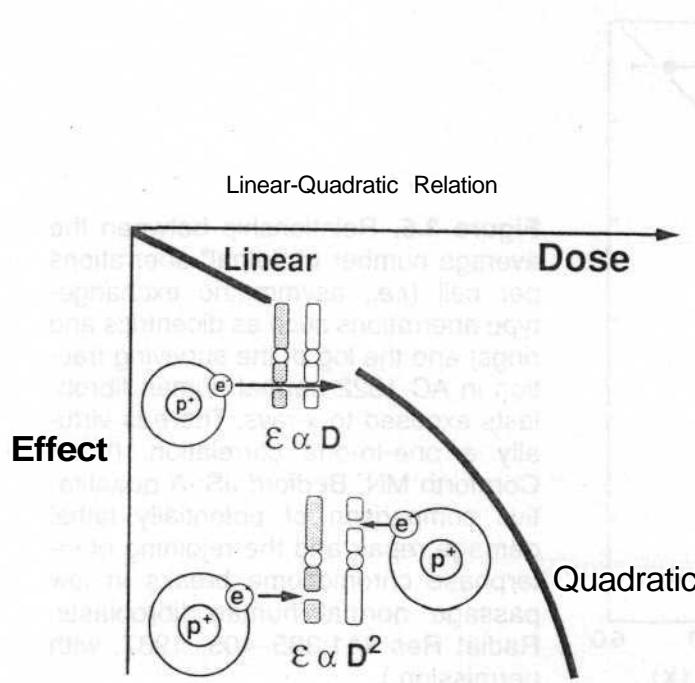
Data such as these provide strong circumstantial evidence to support the notion that asymmetrical exchange-type aberrations represent the principle mechanism for radiation-induced mitotic death in mammalian cells.

Figure 3.6 illustrates, in a much oversimplified way, the relationship between chromosome aberrations and cell killing. As explained in Chapter 2, cells in which there is an asymmetric exchange-type aberration (such as a dicentric or a ring) lose their reproductive integrity. Exchange type aberrations require two chromosome breaks. At low doses, the two breaks may result from the passage of a single electron set in motion by the absorption of a photon of x- or y-rays. The probability of an interaction between the two breaks to form a lethal exchange-type aberration is proportional to dose. Consequently, the survival curve is linear at low doses. At higher doses

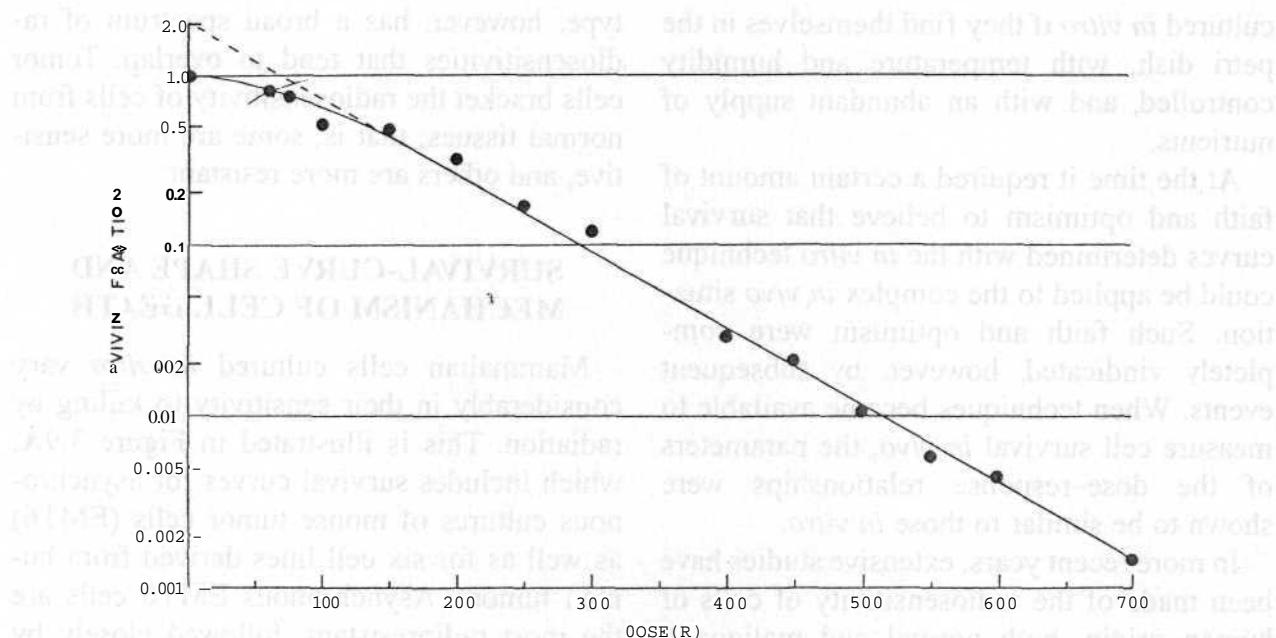
the two chromosome breaks may result from two *separate* electrons. The probability of an interaction between the two breaks is then proportional to the square of the dose. If this quadratic component dominates, the survival curve bends over and becomes curved. Thus, the linear-quadratic relationship characteristic of the induction of chromosome aberrations is carried over to the cell-survival curve.

### SURVIVAL CURVES FOR VARIOUS MAMMALIAN CELLS IN CULTURE

Survival curves have been measured for many established cell lines grown in culture. These cell lines have been derived from the tissues of humans or other mammals, such as small rodents. In some cases the parent tissue has been neoplastic, and in other cases it has been normal. The first *in vitro* survival curve for mammalian cells irradiated with x-rays is shown in Figure 3.7. All mammalian cells studied to date, normal or malignant, regardless of their species of origin exhibit x-ray survival curves similar to those in Figure 3.5; there is an initial shoulder followed by a portion that tends to become straight on a log-linear plot. The size of the initial shoulder is extremely variable. For some cell lines the survival curve appears to bend continuously,



**Figure 3.6.** Relationship between chromosome aberrations and cell survival. Cells that suffer exchange-type chromosome aberrations (such as dicentric) are unable to survive and continue to divide indefinitely. At low doses, the two chromosome breaks are the consequence of a single electron set in motion by the absorption of x- or y-rays. The probability of an interaction between the breaks is proportional to dose; this is the linear portion of the survival curve. At higher doses, the two chromosome breaks may result also from two separate electrons. The probability of an interaction is then proportional to the square of the dose. The survival curve bends if the quadratic component dominates.



**Figure 3.7.** Survival curve for HeLa cells in culture exposed to x-rays. Characteristically, this cell line has a small initial shoulder. (From Puck TT, Markus PI: Action of x-rays on mammalian cells. J Exp Med 103:653-666, 1956, with permission.)

so that the linear-quadratic relationship is a better fit and  $n$  has no meaning. The Do of the x-ray survival curves for most cells cultured *in vitro* fall in the range of 1 to 2 Gy (100 to 200 rad). The exceptions are cells from patients with cancer-prone syndromes, such as ataxia telangiectasia (AT); these cells are much more sensitive to ionizing radiations, with a Do for x-rays of about 0.5 Gy (50 rad). This *in vitro* sensitivity correlates with a hypersensitivity to radiotherapy found in these persons.

The first *in vitro* survival curve was reported in 1956 and generated great excitement in the field of radiobiology. It was thought that at last, with a quantitative system available to relate absorbed dose with surviving fraction of cells, great strides would be made in understanding the effect of ionizing radiation on biologic materials. In particular, it was anticipated that significant contributions would be made toward understanding radiotherapeutic practice. This enthusiasm was not shared by everyone. Some researchers were skeptical that these *in vitro* techniques, which involved growing cells in petri dishes in very artificial conditions, would ever bene-

fit clinical radiotherapy. The fears of these skeptics were eloquently voiced by F. G. Spear in the MacKenzie Davidson Memorial Lecture given to the British Institute of Radiology in 1957:

An isolated cell *in vitro* does not necessarily behave as it would have done if left *in vivo* in normal association with cells of other types. Its reactions to various stimuli, including radiations, however interesting and important in themselves, may indeed be no more typical of its behavior in the parent tissue than Robinson Crusoe on his desert island was representative of social life in York in the mid-seventeenth century.

The appropriate answer to this charge was given by David Gould, then professor of radiology at the University of Colorado. He pointed out that the *in vitro* culture technique measured the reproductive integrity of cells, and that there was no reason to suppose that Robinson Crusoe's reproductive integrity was any different on his desert island from what it would have been had he remained in York; all that Robinson Crusoe lacked was the opportunity. The opportunity to reproduce to the limit of their capability is afforded to cells

cultured *in vitro* if they find themselves in the petri dish, with temperature and humidity controlled, and with an abundant supply of nutrients.

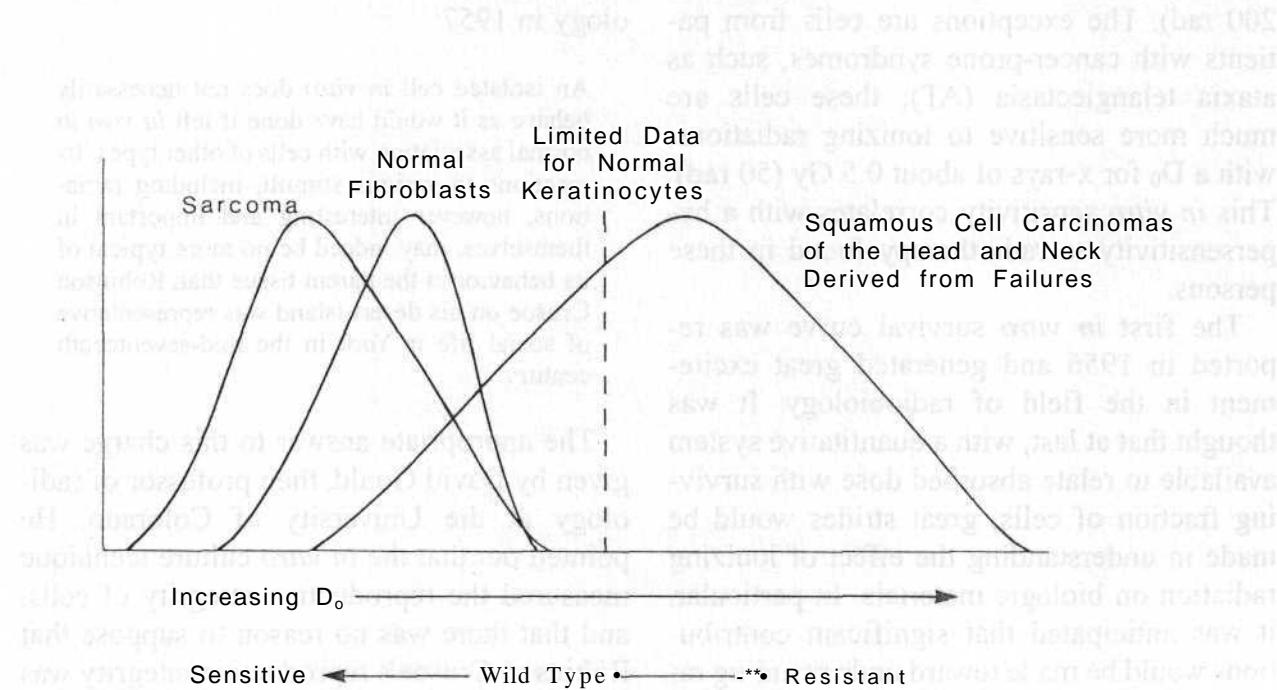
At the time it required a certain amount of faith and optimism to believe that survival curves determined with the *in vitro* technique could be applied to the complex *in vivo* situation. Such faith and optimism were completely vindicated, however, by subsequent events. When techniques became available to measure cell survival *in vivo*, the parameters of the dose-response relationships were shown to be similar to those *in vitro*.

In more recent years, extensive studies have been made of the radiosensitivity of cells of human origin, both normal and malignant, grown and irradiated in culture. In general, cells from a given normal tissue show a narrow range of radiosensitivities if many hundreds of persons are studied (Fig. 3.8). By contrast, cells from human tumors show a very broad range of  $D_0$  values; some cells such as those from squamous carcinomas tend to be more radioresistant, and sarcomas are somewhat more radiosensitive. Each tumor

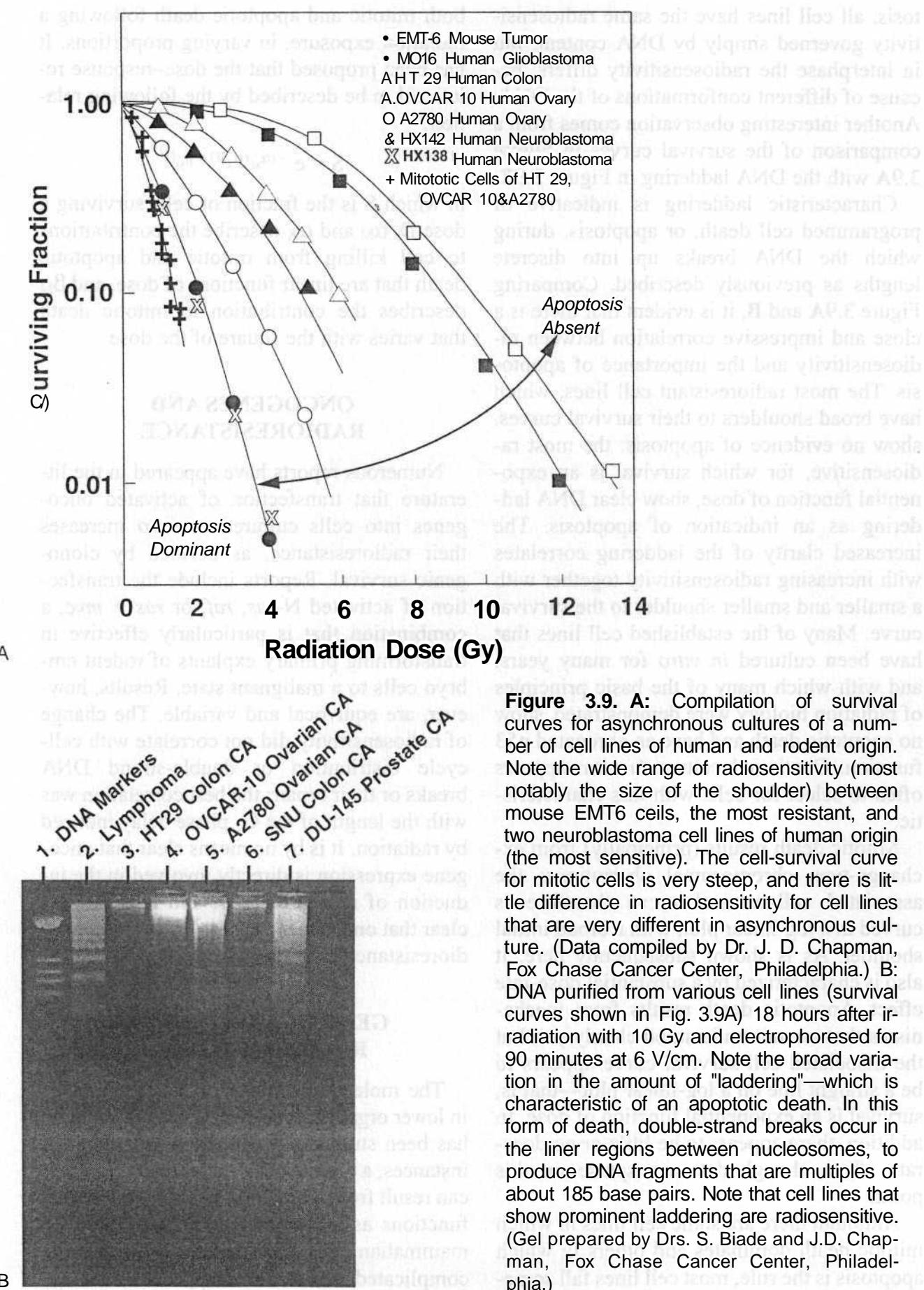
type, however, has a broad spectrum of radiosensitivities that tend to overlap. Tumor cells bracket the radiosensitivity of cells from normal tissues; that is, some are more sensitive, and others are more resistant.

### SURVIVAL-CURVE SHAPE AND MECHANISM OF CELL DEATH

Mammalian cells cultured *in vitro* vary considerably in their sensitivity to killing by radiation. This is illustrated in Figure 3.9A, which includes survival curves for asynchronous cultures of mouse tumor cells (EMT6) as well as for six cell lines derived from human tumors. Asynchronous EMT6 cells are the most radioresistant, followed closely by glioblastoma cells of human origin; thereafter radiosensitivity increases, with two neuroblastoma cell lines being the most sensitive. Although asynchronous cells show this wide range of sensitivities to radiation, it is a remarkable finding that mitotic cells from all of these cell lines have essentially the same radiosensitivity. The implication of this is that, if the chromosomes are condensed during mi-



**Figure 3.8.** Summary of  $D_0$  values for cells of human origin grown and irradiated *in vitro*. Cells from human tumors tend to have a wide range of radiosensitivities, which brackets the radiosensitivity of normal human fibroblasts. In general, squamous-cell carcinoma cells are more resistant than sarcoma cells, but the spectra of radiosensitivities are broad and overlap. (Courtesy of Dr. Ralph Wechslerbaum.)



**Figure 3.9.** A: Compilation of survival curves for asynchronous cultures of a number of cell lines of human and rodent origin. Note the wide range of radiosensitivity (most notably the size of the shoulder) between mouse EMT6 cells, the most resistant, and two neuroblastoma cell lines of human origin (the most sensitive). The cell-survival curve for mitotic cells is very steep, and there is little difference in radiosensitivity for cell lines that are very different in asynchronous culture. (Data compiled by Dr. J. D. Chapman, Fox Chase Cancer Center, Philadelphia.) B: DNA purified from various cell lines (survival curves shown in Fig. 3.9A) 18 hours after irradiation with 10 Gy and electrophoresed for 90 minutes at 6 V/cm. Note the broad variation in the amount of "laddering"—which is characteristic of an apoptotic death. In this form of death, double-strand breaks occur in the linker regions between nucleosomes, to produce DNA fragments that are multiples of about 185 base pairs. Note that cell lines that show prominent laddering are radiosensitive. (Gel prepared by Drs. S. Biade and J.D. Chapman, Fox Chase Cancer Center, Philadelphia.)

tosis, all cell lines have the same radiosensitivity governed simply by DNA content, but in interphase the radiosensitivity differs, because of different conformations of the DNA. Another interesting observation comes from a comparison of the survival curves in Figure 3.9A with the DNA laddering in Figure 3.9B.

Characteristic laddering is indicative of programmed cell death, or apoptosis, during which the DNA breaks up into discrete lengths as previously described. Comparing Figure 3.9A and B, it is evident that there is a close and impressive correlation between radiosensitivity and the importance of apoptosis. The most radioresistant cell lines, which have broad shoulders to their survival curves, show no evidence of apoptosis; the most radiosensitive, for which survival is an exponential function of dose, show clear DNA laddering as an indication of apoptosis. The increased clarity of the laddering correlates with increasing radiosensitivity together with a smaller and smaller shoulder to the survival curve. Many of the established cell lines that have been cultured *in vitro* for many years, and with which many of the basic principles of radiation biology were demonstrated, show no apoptotic death and have an abrogated p53 function. Continued culture *in vitro* appears often to select for cells with this characteristic.

Mitotic death results (principally) from exchange-type chromosomal aberrations; the associated cell-survival curve therefore is curved in a log-linear plot, with a broad initial shoulder. As is shown subsequently here, it also is characterized by a substantial dose-rate effect. Apoptotic death results from mechanisms that are not understood clearly yet, but the associated cell-survival curve appears to be a straight line on a log-linear plot—that is, survival is an exponential function of dose. In addition, there appears to be little or no dose-rate effect, though data are sparse on this point.

Although there are some cell lines in which mitotic death dominates and others in which apoptosis is the rule, most cell lines fall somewhere in between, with contributions from

both mitotic and apoptotic death following a radiation exposure, in varying proportions. It has been proposed that the dose-response relationship be described by the following relation:

$$S = e^{-(\alpha_M + \alpha_A)L_{\gamma} P M^D}$$

in which S is the fraction of cells surviving a dose D,  $\alpha_M$  and  $\alpha_A$  describe the contributions to cell killing from mitotic and apoptotic death that are linear functions of dose, and  $(3M)$  describes the contribution to mitotic death that varies with the square of the dose.

## ONCOGENES AND RADIORESISTANCE

Numerous reports have appeared in the literature that transfection of activated oncogenes into cells cultured *in vitro* increases their radioreistance, as defined by clonogenic survival. Reports include the transfection of activated **N-ius**, **raf**, or **ras + myc**, a combination that is particularly effective in transforming primary explants of rodent embryo cells to a malignant state. Results, however, are equivocal and variable. The change of radiosensitivity did not correlate with cell-cycle distribution or double-strand DNA breaks or their repair; the best correlation was with the length of the G2 phase delay induced by radiation. It is by no means clear that oncogene expression is directly involved in the induction of radioreistance, and it is far less clear that oncogenes play any major role in radioreistance in human tumors.

## GENETIC CONTROL OF RADIOSENSITIVITY

The molecular biology of repair processes in lower organisms, such as yeast and bacteria, has been studied extensively. In a number of instances, a dramatically radiosensitive mutant can result from a mutation in a single gene that functions as a repair or checkpoint gene. In mammalian cells, the situation is much more complicated, and it would appear that a large number of genes may be involved in deter-

mining radiosensitivity. Many radiosensitive mutants have been isolated from cell lines maintained in the laboratory, especially rodent cell systems. In many but not all cases, their sensitivity to cell killing by radiation has been related to their greatly reduced ability to repair double-strand DNA breaks (DSBs). Examples of these genes are XRCC5 Ku 80, XRCC6 Ku 70, and XRCC7. The first of these two genes are involved in DNA-dependent kinase activity that binds to the free ends at the site of a double-strand break, so that if they are defective, repair of double-strand breaks is prejudiced. The third gene codes for a protein that is defective in mice with the "severe combined immune deficiency syndrome" that are sensitive to radiation.

Some patients who show an abnormally severe normal tissue reaction to radiation therapy exhibit the traits of specific inherited syndromes. These are listed in Table 3.1. The most striking example is AT. Fibroblasts taken from patients suffering from this syndrome are two or three times as radiosensitive as normal, and patients with AT receiving radiation therapy show considerable normal tissue damage unless the doses are reduced appropriately. They also have an elevated incidence of spontaneous cancer. Cells from AT heterozygotes are slightly more radiosensitive than normal, and there is some controversy as to whether AT heterozygotes are predisposed to cancer.

The gene associated with AT has been identified and sequenced and called the ATM (AT-mutated) gene. The ATM protein appears to be part of signal transduction pathways involved in many physiologic processes, though

the exact mechanism by which the genetic defect in AT cells leads to radiosensitivity is not altogether clear.

### INTRINSIC RADIOSENSITIVITY AND PREDICTIVE ASSAYS

Predictive assays of individual tumor radiosensitivity require cells to be grown from fresh explants of human tumor biopsies. These do not grow well as attached cells in regular clonogenic assays. Better results have been obtained with the Courtenay assay, in which cells grow in a semisolid agar gel supplemented with growth factors. In addition, a number of nonclonogenic assays have been developed based on cell growth in a multiwell plate. Growth is assessed in terms of the ability of cells to reduce a compound that can be visualized by staining or is based on total DNA or RNA content of the well. These endpoints are surrogates for clonogenicity or reproductive integrity. See Chapter 23 for a discussion of predictive assays.

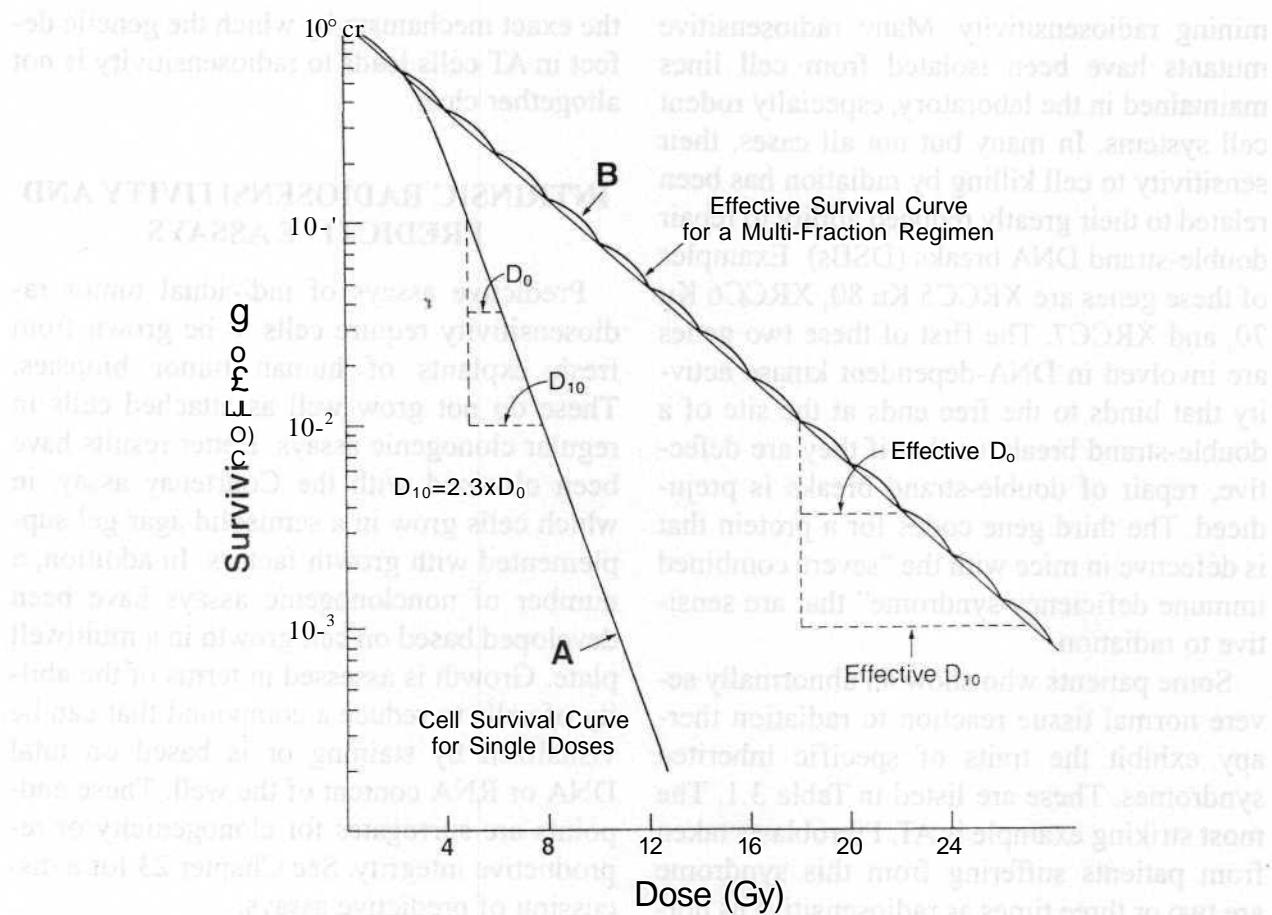
### EFFECTIVE SURVIVAL CURVE FOR A MULTIFRACTION REGIMEN

Because multifraction regimens are used most often in clinical radiotherapy, it is frequently useful to think in terms of an effective survival curve.

If a radiation dose is delivered in a series of equal fractions, separated by sufficient time for repair of sublethal damage to occur between doses, the **effective dose survival curve** becomes an exponential function of dose. The shoulder of the survival curve is repeated many times so that the effective survival curve is a straight line from the origin through a point on the single-dose survival curve corresponding to the daily dose fraction. This is illustrated in Figure 3.10. The effective survival curve is an exponential function of dose whether the single-dose survival curve has a constant terminal slope (as shown) or is continuously bending, as implied by the linear-quadratic relation. The Do of the effective survival curve (*i.e.*, the reciprocal of

TABLE 3.1. *Inherited Human Syndromes Associated with Sensitivity to X-Rays*

Ataxia telangiectasia
Basal cell nevoid syndrome
Cockayne's syndrome
Down syndrome
Fanconi's anemia
Gardner's syndrome
Nijmegen breakage syndrome
Usher's syndrome



**Figure 3.10.** The concept of an "effective" survival curve for a multifraction regimen is illustrated. If the radiation dose is delivered in a series of equal fractions separated by time intervals sufficiently long for the repair of sublethal damage to be complete between fractions, the shoulder of the survival curve is repeated many times. The effective dose-survival curve is an exponential function of dose, that is, a straight line from the origin through a point on the single-dose survival curve corresponding to the daily dose fraction (e.g., 2 Gy). The dose resulting in one decade of cell killing ( $D_{10}$ ) is related to the  $D_0$  by the expression  $D_{10} = 2.3 D_0$ .

the slope), defined to be the dose required to reduce the fraction of cells surviving to 37%, has a value close to 300 cGy for cells of human origin. This is an average value and can differ significantly for different tumor types.

For calculation purposes, it is often useful to use the  $D_{10}$ , the dose required to kill 90% of the population. For example,

$$D_{10}=2.3 \times D_0$$

in which 2.3 is the natural logarithm of 10.

### CALCULATIONS OF TUMOR CELL KILL

The concept outlined previously of an effective survival curve for a multifraction radi-

ation treatment may be used to perform simple calculations of tumor cell kill after radiotherapy. Although such calculations are greatly oversimplified, they are nevertheless instructive. Four examples are given here.

#### Problem 1

A tumor consists of  $10^9$  clonogenic cells. The effective dose-response curve, given in daily dose fractions of 2 Gy, has no shoulder and a  $D_0$  of 3 Gy. What total dose is required to give a 90% chance of tumor cure?

#### Answer

To give a 90% probability of tumor control in a tumor containing  $10^9$  cells requires a cel-

lular depopulation of  $10^{-10}$ . The dose resulting in one decade of cell killing ( $D_{10}$ ) is given by

$$D_{10} = 2.3 \times D_0 = 2.3 \times 3 = 6.9 \text{ Gy}$$

Total dose for 10 decades of cell killing, therefore, is  $10 \times 6.9 = 69 \text{ Gy}$ .

### Problem 2 \*

Suppose that, in the previous example, the clonogenic cells underwent three cell doublings during treatment. About what total dose would be required to achieve the same probability of tumor control?

### Answer

Three cell doublings would increase the cell number by

$$2 \times 2 \times 2 = 8$$

Consequently, about one extra decade of cell killing would be required, corresponding to an additional dose of 6.9 Gy. Total dose is  $69 + 6.9 = 75.9 \text{ Gy}$ .

### Problem 3

During the course of radiotherapy, a tumor containing  $10^9$  cells receives 40 Gy. If the  $D_0$  is 2.2 Gy, how many tumor cells will be left?

### Answer

If the  $D_0$  is 2.2 Gy the  $D_{10}$  is given by

$$\begin{aligned} D_{10} &= 2.3 \times D_0 \\ &= 2.3 \times 2.2 = 5 \text{ Gy} \end{aligned}$$

Because the total dose is 40 Gy, the number of decades of cell killing is  $40/5 = 8$ .

Number of cells remaining =  $10^9 \times 10^{-8} = 10$

### Problem 4

If  $10^7$  cells were irradiated according to single-hit kinetics so that the average number of hits per cell is one, how many cells would survive?

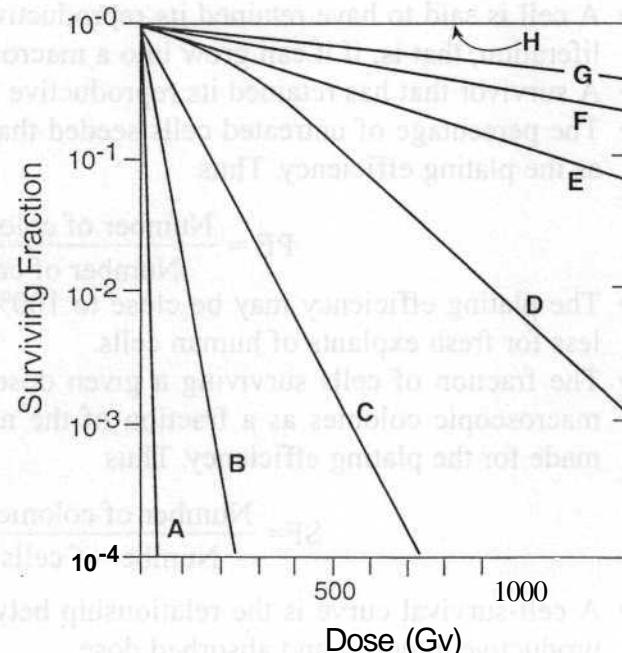
### Answer

A dose that gives an average of one hit per cell is the  $D_0$ ; that is, the dose that on the exponential region of the survival curve reduces the number of survivors to 37%; the number of surviving cells therefore is

$$10^7 \times \frac{37}{100} = 3.7 \times 10^6$$

## THE RADIOSENSITIVITY OF MAMMALIAN CELLS COMPARED WITH MICROORGANISMS

The final illustration in this chapter (Fig. 3.11) is a compilation from the literature of survival data for many types of cells. The steepest dose-response relationship (curve A) is an average curve for mammalian cells; it is evident that they are exquisitely radiosensitive compared with micro-organisms. The bacteria *Escherichia coli* are more resistant, yeast is



**Figure 3.11.** Survival curves for mammalian cells and for a variety of microorganisms, including *Escherichia coli*, yeast, and *Micrococcus radiodurans*. It is evident that mammalian cells are exquisitely radiosensitive compared with microorganisms, principally because they have a much larger DNA content, which represents a bigger "target" for radiation damage. A, mammalian cells; B, *E. coli*; C, *E. coli* B/r; D, yeast; E, phage staph E; F, *B. megatherium*; G, potato virus; H, *Micrococcus radiodurans*.

more resistant still, and the most resistant is *Micrococcus radiodurans*, which shows no significant cell killing even after a dose of **1,000 Gy** (105 rad). There are several important points to be made from this:

1. The dominant factor that accounts for this huge range of radiosensitivities is the DNA content. Mammalian cells are sensitive because they have a large DNA content, which represents a large target for radiation damage.
2. DNA content is not the whole story, however. *E. coli* and *E. coli* B/r have the same
3. Figure 3.11 explains why, if radiation is used as a method of sterilization, doses of the order of 20,000 Gy (2 million rad) are necessary. Even if objects are socially clean, such huge doses are necessary to reduce the population of contaminating microorganisms, because of their extreme radioresistance.

### SUMMARY OF PERTINENT CONCLUSIONS

- Cells from tumors and many normal regenerative tissues grow and form colonies *in vitro*.
- Fresh explants of normal tissues often grow well in culture for a few weeks before they peter out and die. A few pass through a "crisis" and become immortal; these are the established cell lines.
- A cell is said to have retained its reproductive integrity if it is capable of sustained proliferation, that is, if it can grow into a macroscopic colony.
- A survivor that has retained its reproductive integrity is said to be *clonogenic*.
- The percentage of untreated cells seeded that grow into macroscopic colonies is known as the plating efficiency. Thus

$$PE = \frac{\text{Number of colonies counted}}{\text{Number of cells seeded}} \times 100$$

- The plating efficiency may be close to 100% for some established cell lines but 1% or less for fresh explants of human cells.
- The fraction of cells surviving a given dose is determined by counting the number of macroscopic colonies as a fraction of the number of cells seeded. Allowance must be made for the plating efficiency. Thus

$$SF = \frac{\text{Number of colonies counted}}{\text{Number of cells seeded}} \times PE/100$$

- A cell-survival curve is the relationship between the fraction of cells retaining their reproductive integrity and absorbed dose.
- Conventionally, surviving fraction on a logarithmic scale is plotted on the ordinate against dose on the abscissa. The shape of the survival curve is important.
- The cell-survival curve for  $\alpha$ -particles and low-energy neutrons (densely ionizing radiations) is a straight line on a log-linear plot; that is, survival approximates to an exponential function of dose.
- The cell-survival curve for x- or y-rays (sparsely ionizing radiations) has an initial slope, followed by a bending region or shoulder, after which it tends to straighten again at higher doses.

DNA content but differ in radiosensitivity because B/r has a mutant, and more efficient, DNA repair system. In higher organisms, mode of cell death, that is, apoptotic versus mitotic, also affects radiosensitivity.

- Survival data are adequately fitted by many models and theories. The data are never sufficiently precise nor are the models sufficiently different for experimental results to discriminate among models.
- For the first one or two decades of survival and up to doses used in single fractions in radiotherapy, survival data are adequately represented by the linear-quadratic relationship

$$S = e^{\alpha D - \beta D^2}$$

in which  $S$  is the fraction of cells surviving a dose  $D$  and  $\alpha$  and  $\beta$  are constants representing the linear and quadratic components of cell killing.

- The initial slope of the cell-survival curve is determined by  $\alpha$ ; the quadratic component of cell killing ((3)) causes the curve to bend at higher doses.
- The ratio  $\alpha/\beta$  is the dose at which linear and quadratic components of cell killing are equal.
- There is good evidence that the nucleus, specifically the DNA, is the principal target for radiation-induced cell lethality. Membrane damage also may be a factor.
- Following exposure to radiation, cells may die attempting the next or a subsequent mitosis (mitotic death), or they may die programmed cell deaths (apoptotic death).
- In cells that die a mitotic death, there is a one-to-one correlation between cell survival and the average number of putative "lethal" chromosomal aberrations per cell, that is, asymmetric exchange-type aberrations such as dicentrics and rings.
- Cells that die apoptotic deaths follow a stereotyped sequence of morphologic events, culminating in the breaking up of the DNA into fragments that are multiples of 185 base pairs; this leads to the characteristic DNA laddering seen in gels.
- In some cell types (such as lymphoid cells) apoptotic death is dominant following irradiation. Survival is then an exponential function of dose; that is, the survival curve is straight and shoulderless on the usual log-linear plot. There is also no dose-rate effect.
- In some cell types (such as CHO or V79 cells in culture) mitotic death is dominant following irradiation. Survival is then a linear-quadratic function of dose; that is, the survival curve has a shoulder on the usual log-linear plot. There is usually a large dose-rate effect.
- Many cell populations die both mitotic and apoptotic deaths. There is, in general, a correlation between the importance of apoptosis and radiosensitivity. If apoptosis is dominant, cells are radiosensitive; if apoptosis is absent, cells are radioresistant.
- Cells cultured from different tumors in humans show a broad range of radiosensitivities that bracket the sensitivity of normal cells from different persons.
- There is some evidence in cells cultured *in vitro* that transfection of activated oncogenes in cells increases their radioresistance. It is not clear that oncogenes play a role in the resistance of human tumors *in vivo*.
- A number of genes that influence the radiosensitivity of mammalian cells have been identified.
- If these genes are defective, the repair of double-strand breaks is often prejudiced.
- A number of human syndromes have been found to be associated with radiosensitivity; AT is the best example.
- There is often a link between sensitivity to killing by radiation and predisposition to cancer.
- Predictive assays for intrinsic radiosensitivity of tumor cells from individual patients require special assays. This is discussed in Chapter 23.

- The effective survival curve for a multifraction regimen is an exponential function of dose: a straight line from the origin through a point on the single-dose survival curve corresponding to the daily dose fraction.
- The average value of the effective  $D_0$  for the multifraction survival curve for human cells is about 3 Gy.
- The  $D_{10}$ , the dose resulting in one decade of cell killing, is related to the  $D_0$  by the expression

$$D_{10} = 2.3 \times D_0$$

- Calculations of tumor-cell kill can be performed for fractionated clinical radiotherapy regimens using the concept of effective survival curve.
- Mammalian cells are exquisitely radiosensitive compared with microorganisms such as bacteria and yeast, principally because of their larger DNA content, which represents a bigger "target" for radiation damage.

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## Radiosensitivity and Cell Age in the Mitotic Cycle

THE CELL CYCLE  
SYNCHRONOUSLY DIVIDING CELL  
CULTURES  
THE EFFECT OF X-RAYS ON  
SYNCHRONOUSLY DIVIDING  
CELL CULTURES  
MOLECULAR CHECKPOINT  
GENES  
THE EFFECT OF OXYGEN AT VARIOUS  
PHASES OF THE CELL CYCLE

THE AGE-RESPONSE FUNCTION FOR A  
TISSUE *IN VIVO*  
VARIATION OF SENSITIVITY WITH CELL  
AGE FOR NEUTRONS  
MECHANISMS FOR THE AGE-RESPONSE  
FUNCTION  
THE POSSIBLE IMPLICATIONS OF THE  
AGE-RESPONSE FUNCTION IN  
RADIOTHERAPY  
SUMMARY OF PERTINENT CONCLUSIONS

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### THE CELL CYCLE

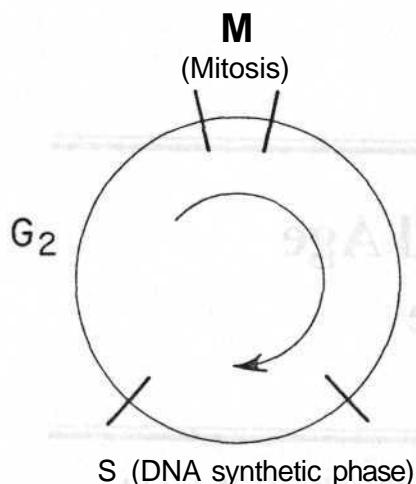
Mammalian cells propagate and proliferate by mitosis. When a cell divides, two progeny cells are produced, each of which carries a chromosome complement identical to that of the parent cell. After an interval of time has elapsed, each of the progeny may undergo a further division. The time between successive divisions is known as the mitotic-cycle time, or, as it is commonly called, the cell-cycle time.

If a population of dividing cells is observed with a conventional light microscope, the only event in the entire cell cycle that can be identified and distinguished is mitosis or division itself. Just before the cell divides to form two progeny, the chromosomes (which are diffuse and scattered in the cell in the period between mitoses) condense into clearly distinguishable forms. In addition, in monolayer cultures of cells, just before mitosis, the cells round up and become loosely attached to the surface of the culture vessel. This whole process of mitosis—in preparation for which the cell

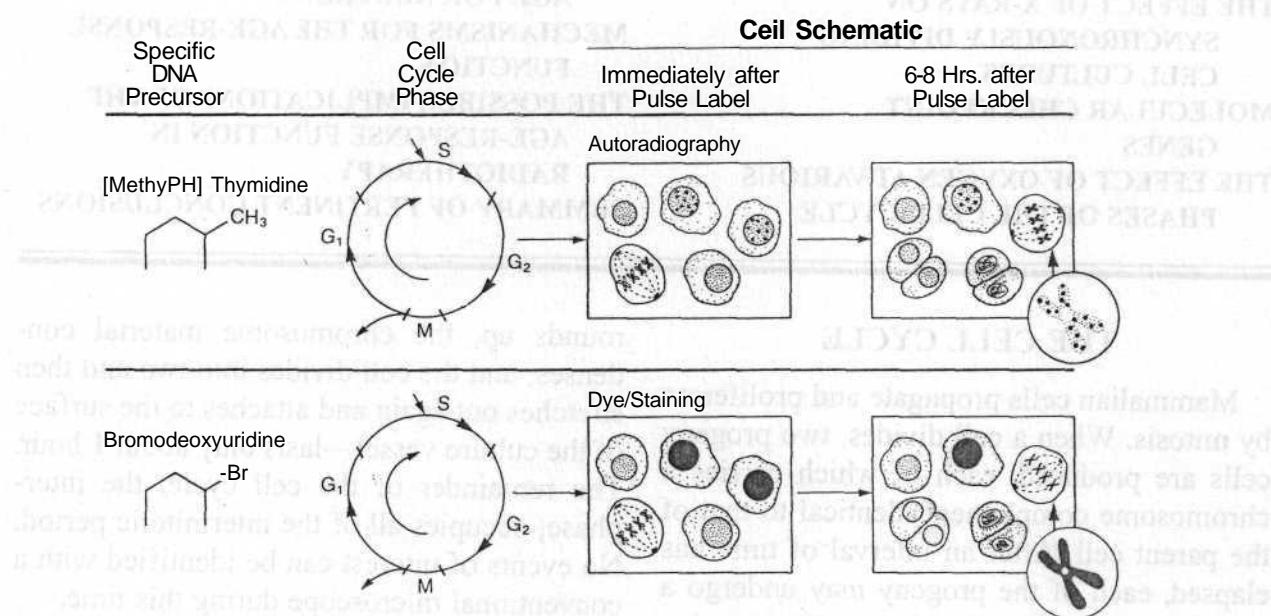
rounds up, the chromosome material condenses, and the cell divides into two and then stretches out again and attaches to the surface of the culture vessel—lasts only about 1 hour. The remainder of the cell cycle, the interphase, occupies all of the intermitotic period. No events of interest can be identified with a conventional microscope during this time.

Because cell division is a cyclic phenomenon, repeated in each generation of the cells, it is usual to represent it as a circle, as shown in Figure 4.1. The circumference of the circle represents the full mitotic cycle time for the cells ( $T_c$ ); the period of mitosis is represented by  $M$ . The remainder of the cell cycle can be further subdivided by using some marker of DNA synthesis. The original technique was autoradiography, introduced by Howard and Pelcin 1953.

The basis of the technique, illustrated in Figure 4.2, is to feed the cells thymidine, a basic building block used for making DNA, which has been labeled with radioactive tritiated thymidine ( $^3H$  TdR). Cells that are actively synthesizing new DNA as part of the process



**Figure 4.1.** The stages of the mitotic cycle for actively growing mammalian cells. M, mitosis; S, DNA synthetic phase; G<sub>1</sub> and G<sub>2</sub>, "gaps" or periods of apparent inactivity between the major discernible events in the cycle.

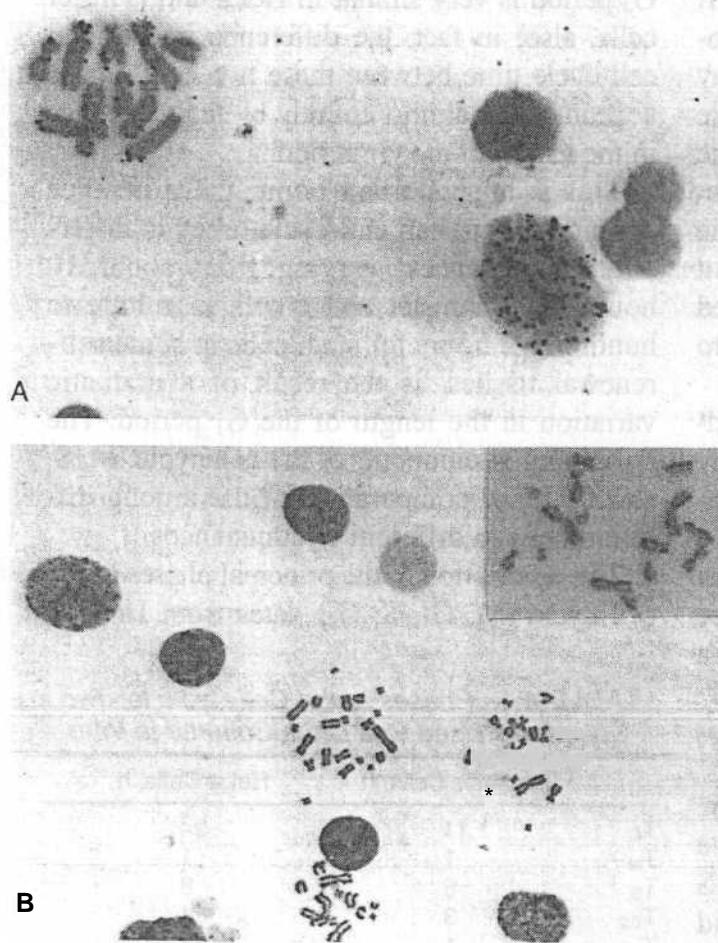


**Figure 4.2.** Cell-labeling techniques. **Left panels:** The principle of autoradiography, which may be applied to cells in culture growing as a monolayer on a glass microscope slide or to thin sections cut from a tumor or normal tissue. Cells in the DNA synthetic phase (S) take up tritiated thymidine. After the cells are fixed and stained so that they are visible by light microscopy, they are covered with a layer of nuclear (photographic) emulsion and left for several weeks in a cool refrigerator. As  $\beta$ -particles from the tritiated thymidine pass through the emulsion, they form latent images that appear as black grains when the emulsion is subsequently developed and fixed. If cells are stained and autoradiographed immediately after incorporation of the tritiated thymidine, cells that are labeled are in S phase. If the staining and autoradiography is delayed for hours after the flash-label (**right panels**), some cells may move from S to M, and labeled mitotic cells are observed. The lengths of the various phases of the cycle can be determined in this way. **Lower panels:** The principle of cell-cycle analysis using 5-bromodeoxyuridine as the DNA precursor instead of radioactively labeled thymidine. The bromodeoxyuridine is incorporated into cells in S. It can be recognized by the use of a specific stain or a monoclonal antibody to bromodeoxyuridine-substituted DNA. The antibody is tagged with a fluorescing dye (e.g., fluorescein), which shows up bright green under a fluorescence microscope. If cells are stained immediately after labeling with bromodeoxyuridine, those staining darkly are in S phase. If staining is delayed for 6 to 8 hours, cells incorporating bromodeoxyuridine may move from S to M, and a darkly staining mitotic cell is seen (**right panel**). (Courtesy of Dr. Charles Geard.)

of replicating their chromosome complements incorporate the radioactive thymidine. The surplus radioactive thymidine then is flushed from the system, the cells are fixed and stained so that they may be viewed easily, and the preparation of cells is coated with a very thin layer of nuclear (photographic) emulsion.

(3-Particles from cells that have incorporated radioactive thymidine pass through the nuclear emulsion and produce a latent image. When the emulsion subsequently is developed and fixed, the area through which a  $\beta$ -particle

has passed appears as a black spot. It is then a comparatively simple matter to view the preparation of cells and to observe that some of the cells have black spots or "grains" over them, which indicates that they were actively synthesizing DNA at the time radioactive thymidine was made available. Other cells do not have any grains over their nuclei; this is interpreted to mean that the cells were not actively making DNA when the radioactive label was made available to them. Examples of labeled cells are shown in Figure 4.3. If the



**Figure 4.3. A:** Autoradiograph of Chinese hamster cells in culture flash-labeled with tritiated thymidine. The black grains over some cells indicate that they were synthesizing DNA when they were labeled. Also shown is a labeled mitotic cell. This cell was in S phase when the culture was flash-labeled but moved to M phase before it was stained and autoradiographed. **B:** Photomicrograph showing cells labeled and unlabeled with bromodeoxyuridine. Cells were grown in the presence of bromodeoxyuridine and then fixed and stained 20 hours later. Incorporated bromodeoxyuridine stains purple, which shows up dark in this black and white print; the rest of the cell is light blue. The stained interphase cell indicates that it was in S phase during the time the bromodeoxyuridine was available. Also shown is a first-generation mitotic cell, which had been in S phase at the time the bromodeoxyuridine was available and had moved to M phase by the time it was fixed and stained. It can be identified as first-generation because both chromatids of each chromosome are stained uniformly. **Inset:** A second-generation mitotic cell, which passed through two S phases during bromodeoxyuridine availability. One chromatid of each chromosome is darker because both strands of the DNA double helix have incorporated bromodeoxyuridine. One chromatid is lighter because only one strand of the DNA has incorporated bromodeoxyuridine. (Courtesy of Dr. Charles Gead.)

cells are allowed to grow for some time after labeling with tritiated thymidine, so that they move into mitosis before being fixed, stained, and autoradiographed, then a labeled mitotic cell may be observed (Fig. 4.3A).

In recent years the use of tritiated thymidine to identify cells in the DNA synthetic phase (S) has been replaced largely with the use of 5-bromodeoxyuridine, which differs from thymidine only by the substitution of a bromine atom for a methyl group. If this halogenated pyrimidine is fed to the cells, it is incorporated into DNA in place of thymidine and its presence can be detected by using an appropriate stain (Fig. 4.2). In a black-and-white print, cells **incorporating** bromodeoxyuridine appear darkly stained. In practice they are easier to recognize because the stain is brightly colored. To identify cells that are in S phase and have incorporated bromodeoxyuridine even more readily, one can use an antibody against bromodeoxyuridine-substituted DNA, which fluoresces brightly under a fluorescence microscope. Examples of stained and unstained cells are shown in Figure 4.3B. If time is allowed between labeling with bromodeoxyuridine and staining, then a cell may move from S to M phase, and a stained mitotic cell is observed (Fig. 4.3B). If the cell is in the first mitosis after bromodeoxyuridine incorporation, both chromatids of each chromosome are equally stained, as shown in the figure, but by the second mitosis, one chromatid is stained darker than the other (illustrated in the inset to Fig. 4.3B).

The use of bromodeoxyuridine has two advantages over conventional autoradiography using tritiated thymidine. First, it does not involve radioactive material. Second, it greatly shortens the time to produce a result, because if cells are coated with emulsion to produce an autoradiograph, they must be stored in a refrigerator for about 1 month to allow  $\beta$ -particles from the incorporated tritium to produce a latent image in the emulsion.

By using either of these techniques it can be shown that cells synthesize DNA only during a discrete well-defined fraction of the cycle, the S phase. There is an interval between mitosis and DNA synthesis in which no label is incorpo-

rated. This first "gap" in activity was named G<sub>i</sub> by Howard and Pelc, and the nomenclature is used today. After DNA synthesis has been completed, there is a second gap before mitosis, G<sub>2</sub>.

All proliferating mammalian cells, whether in culture or growing normally in a tissue, have a cycle of mitosis (M), followed by G<sub>i</sub>, S, and G<sub>2</sub>, after which mitosis occurs again. The relative lengths of these various constituent parts of the cell cycle vary according to the particular cells studied.

The characteristics of two cell lines commonly used for *in vitro* culture are summarized in Table 4.1. HeLa cells have a total cell-cycle time of about 24 hours, which is more than double that of the Chinese hamster cell, which has a cell-cycle time of about 11 hours. Mitosis lasts only a relatively short time, about 1 hour, and is not very different for those two cell lines or for most others. The S phase is 8 hours for HeLa cells and 6 hours for hamster cells; in all cell lines studied in culture or growing *in vivo*, the S phase never exceeds about 15 hours. The G<sub>2</sub> period is very similar in HeLa and hamster cells, also; in fact, the difference in the total cell-cycle time between these two cell lines is accounted for almost entirely by the difference in the length of the G<sub>i</sub> period.

This is an important point: the difference among mammalian cell-cycle times in different circumstances, varying from about 10 hours for a hamster cell grown in culture to hundreds of hours for stem cells in some self-renewal tissues, is the result of a dramatic variation in the length of the G<sub>i</sub> period. The remaining components of the cell cycle, M, S, and G<sub>2</sub>, vary comparatively little among different cells in different circumstances.

The description of the principal phases of the cell cycle (M, G<sub>i</sub>, S, G<sub>2</sub>) dates from Howard

TABLE 4.1. *Phases of the Cell Cycle for Two Commonly Used Cell Lines Cultured in Vitro*

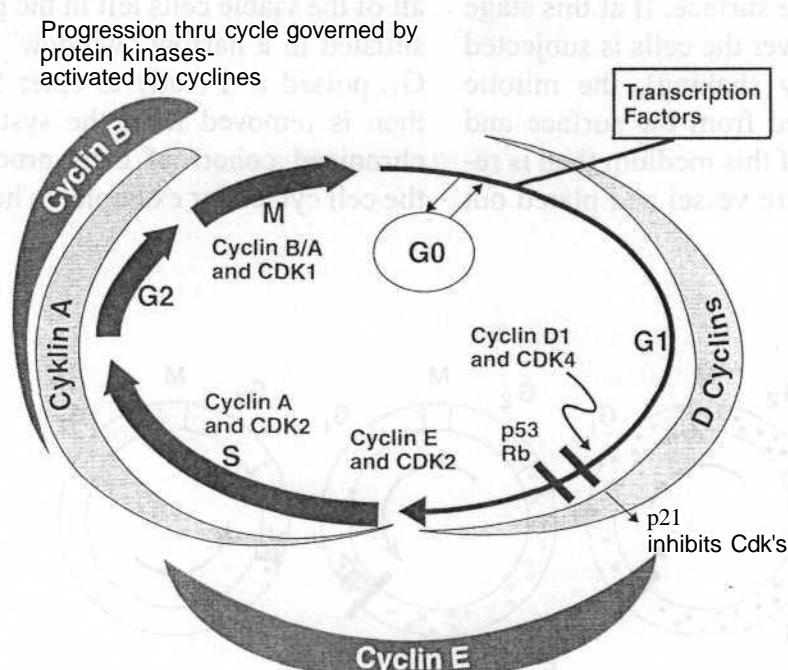
	Hamster Cells, h	HeLa Cells, h
T <sub>c</sub>	11	24
T <sub>M</sub>	1	1
T <sub>R</sub>	6	8
T <sub>G2</sub>	3	4
T <sub>GI</sub>	1	11

and Pelc in 1953 as previously discussed. During a complete cell cycle, the cell must accurately replicate the DNA once during S phase and distribute an identical set of chromosomes equally to two progeny cells during M phase. In recent years we have learned much more about the mechanisms by which the cycle is regulated in eukaryotic cells. Regulation occurs\* by the periodic activation of different members of the cyclin-dependent kinase (CDK) family. In its active form, each CDK is complexed with a particular cyclin. Different CDK—cyclin complexes are required to phosphorylate a number of protein substrates, which drive such cell-cycle events as the initiation of DNA replication or the onset of mitosis. CDK-cyclin complexes are also vital in preventing the initiation of a cell-cycle event at the wrong time.

Extensive regulation of CDK—cyclin activity, by a number of transcriptional and post-transcriptional mechanisms, ensures perfect timing and coordination of cell-cycle events. The CDK catalytic subunit by itself is inactive, requiring association with a cyclin subunit and phosphorylation of a key threonine

residue (usually T-160) to become fully active. The CDK-cyclin complex is reversibly inactivated either by phosphorylation on a tyrosine residue (usually Y-15) located in the adenosine triphosphate-binding domain, or by association with cyclin-kinase inhibitory proteins. After the completion of the cell-cycle transition, the complex is inactivated irreversibly by ubiquitin-mediated degradation of the cyclin subunit.

Entry into S phase is controlled by CDKs that are sequentially regulated by cyclins D, E, and A. D-type cyclins act as growth-factor sensors, with their expression depending more on the extracellular cues than on the cell's position in the cycle. Mitogenic stimulation governs both their synthesis and complex formation with CDK4 and CDK6, and catalytic activity of the assembled complexes persists through the cycle as long as mitogenic stimulation continues. Cyclin E expression in proliferating cells is normally periodic and maximal at the G<sub>1</sub>-S transition, and throughout this interval it enters into active complexes with its catalytic partner, CDK2. Figure 4.4 illustrates this view



**Figure 4.4.** Update of the phases of the cell cycle, showing how they are regulated by the periodic activation of different members of the cyclin-dependent kinase family. Various cyclin-dependent kinase-cyclin complexes are required to phosphorylate a number of protein substrates, which drive key events including the initiation of DNA replication and the onset of mitosis.

of the cell cycle and its regulation. This is in essence an update of Figure 4.1.

### SYNCHRONOUSLY DIVIDING CELL CULTURES

In the discussion of survival curves in Chapter 3 the assumption was implicit that the population of irradiated cells was asynchronous; that is, it consisted of cells distributed throughout all phases of the cell cycle. A study of the variation of radiosensitivity with the position or age of the cell in the cell cycle was made possible only by the development of techniques to produce synchronously dividing cell cultures—populations of cells in which all of the cells occupy the same phase of the cell cycle at a given time.

There are essentially two techniques that have been used to produce a synchronously dividing cell population. The first is the **mitotic harvest** technique, first described by Terasima and Tolmach. This technique can be used only for cultures that grow in monolayers attached to the surface of the growth vessel. It exploits the fact that if such cells are close to mitosis, they round up and become loosely attached to the surface. If at this stage the growth medium over the cells is subjected to gentle motion (by shaking), the mitotic cells become detached from the surface and float in the medium. If this medium then is removed from the culture vessel and plated out

into new petri dishes, the population consists almost entirely of mitotic cells. Incubation of these cell cultures at 37°C then causes the cells to move together synchronously in step through their mitotic cycles. By delivering a dose of radiation at various times after the initial harvesting of mitotic cells, one can irradiate cells at various phases of the cell cycle.

An alternative method for synchronizing cells, which is applicable to cells in a tissue as well as cells grown in culture, involves the use of a drug. A number of different substances may be used. One of the most widely applicable is hydroxyurea. If this drug is added to a population of dividing cells, it has two effects on the cell population. First, all cells that are synthesizing DNA take up the drug and are killed. Second, the drug imposes a block at the end of the G<sub>i</sub> period; cells that occupy the G<sub>2</sub>, M, and G<sub>i</sub> compartments when the drug is added progress through the cell cycle and accumulate at this block.

The dynamics of the action of hydroxyurea are illustrated in Figure 4.5. The drug is left in position for a period equal to the combined lengths of G<sub>2</sub>, M, and G<sub>i</sub> for that particular cell line. By the end of the treatment period, all of the viable cells left in the population are situated in a narrow "window" at the end of G<sub>i</sub>, poised and ready to enter S. If the drug then is removed from the system, this synchronized cohort of cells proceeds through the cell cycle. For example, in hamster cells, 5

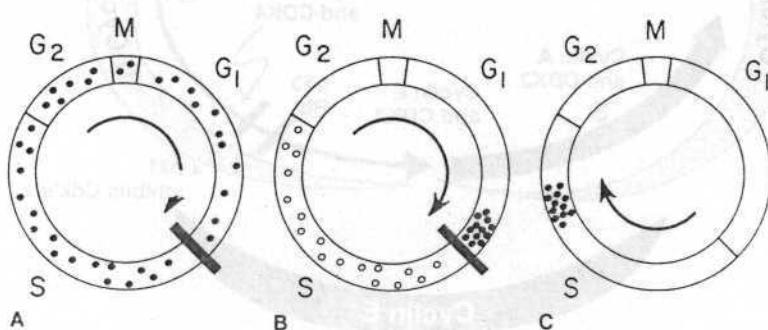


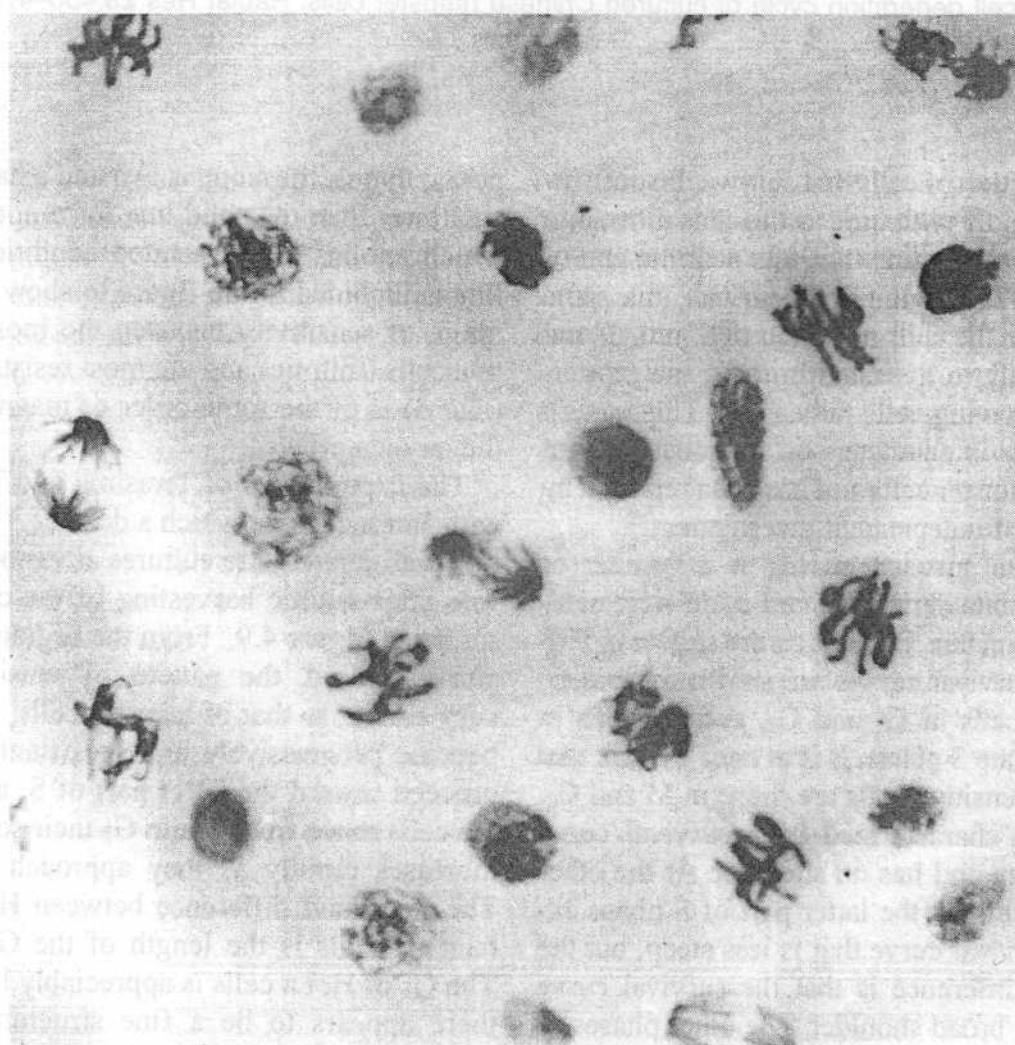
Figure 4.5. Mode of action of hydroxyurea as an agent to induce synchrony. This drug kills cells in S phase and imposes a "block" at the end of G<sub>i</sub> phase. Cells in G<sub>2</sub>, M, and G<sub>i</sub> phases accumulate at this block if the drug is added. If the block is removed, the synchronized cohort of cells moves on through the cycle.

hours after the removal of the drug, the cohort of synchronized cells occupies a position late in the S phase. Some 9 hours after the removal of the drug, the cohort of cells is at, or close to, mitosis.

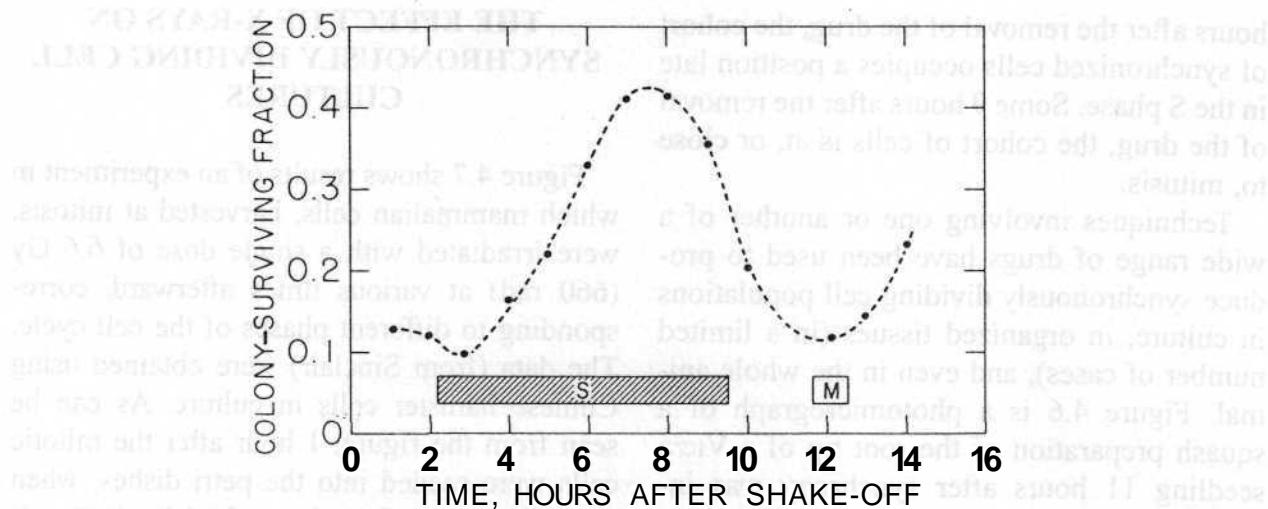
Techniques involving one or another of a wide range of drugs have been used to produce synchronously dividing cell populations in culture, in organized tissues (in a limited number of cases), and even in the whole animal. Figure 4.6 is a photomicrograph of a squash preparation of the root tip of a *Vicia* seedling 11 hours after synchrony was induced with hydroxyurea. A very large proportion of the cells is in mitosis.

### THE EFFECT OF X-RAYS ON SYNCHRONOUSLY DIVIDING CELL CULTURES

Figure 4.7 shows results of an experiment in which mammalian cells, harvested at mitosis, were irradiated with a single dose of 6.6 Gy (660 rad) at various times afterward, corresponding to different phases of the cell cycle. The data (from Sinclair) were obtained using Chinese hamster cells in culture. As can be seen from the figure, 1 hour after the mitotic cells were seeded into the petri dishes, when the cells were in Gi, a dose of 6.6 Gy (660 rad) resulted in a surviving fraction of about 13%.



**Figure 4.6.** Photomicrograph of a squash preparation of the root tip of a *Vicia* seedling 11 hours after synchrony was induced with hydroxyurea. Note the large proportion of cells in mitosis. (From Hall EJ, Brown JM, Cavanagh J: Radiosensitivity and the oxygen effect measured at different phases of the mitotic cycle using synchronously dividing cells of the root meristem of *Vicia faba*. Radiat Res 35:622-634, 1968, with permission)



**Figure 4.7.** Fraction of Chinese hamster cells surviving a dose of 6.6 Gy (660 rad) of x-rays as a function of time. Time zero corresponds to the harvesting of mitotic cells. The cell-surviving fraction increases to a maximum late in S phase. (Adapted from Sinclair WK, Morton RA: X-ray sensitivity during the cell generation cycle of cultured Chinese hamster cells. Radiat Res 29:450-474, 1966, with permission.)

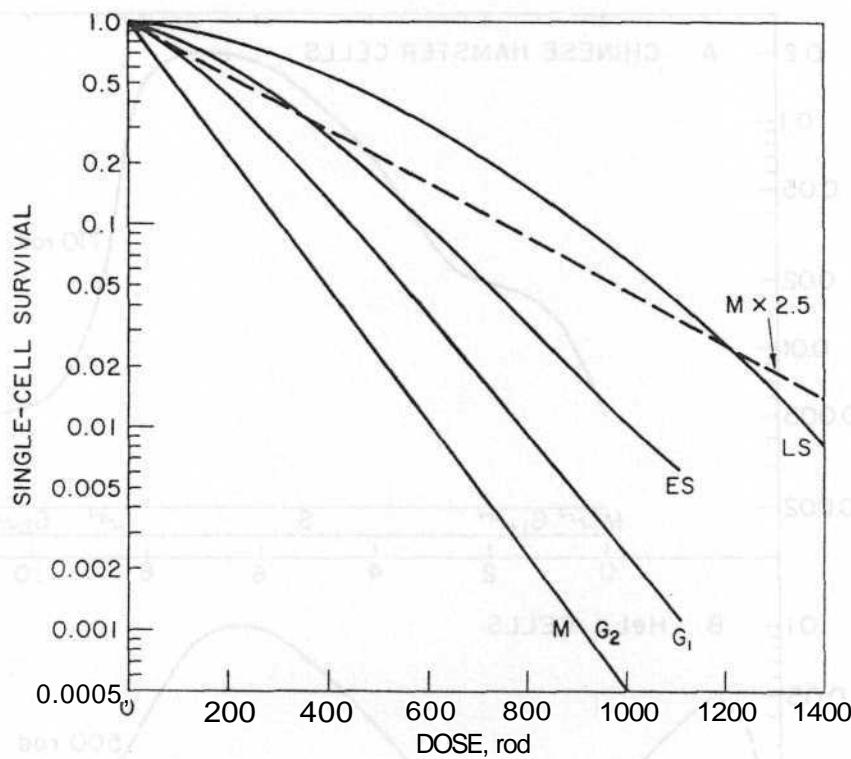
The proportion of cells that survive the dose increases rapidly with time as the cells move into S phases; by the time the cells near the end of S phase, 42% of the cells survive this same dose. When the cells move out of S into G<sub>2</sub> and subsequently to a second mitosis, the proportion of surviving cells falls again. This pattern of response is characteristic for most lines of Chinese hamster cells and has been reported by a number of independent investigators.

Complete survival curves at a number of discrete points during the cell cycle were measured by Sinclair. The results are shown in Figure 4.8. Survival curves are shown for mitotic cells, for cells in G<sub>1</sub> and G<sub>2</sub>, and for cells in early and late S phase. It is at once evident that the most sensitive cells are those in M and G<sub>2</sub>, which are characterized by a survival curve that is steep and has no shoulder. At the other extreme, cells in the latter part of S phase exhibit a survival curve that is less steep, but the essential difference is that the survival curve has a very broad shoulder. The other phases of the cycle, such as G<sub>1</sub> and early S, are intermediate in sensitivity between the two extremes.

The broken line in Figure 4.8 is the calculated survival curve that would be expected to apply for mitotic cells under conditions of hy-

poxia; that is, the slope is two and a half times shallower than the solid line for mitotic cells, which applies to the aerated condition. This line is included in the figure to show that the range of sensitivity between the most sensitive cells (mitotic) and the most resistant cells (late S) is of the same order of magnitude as the oxygen effect.

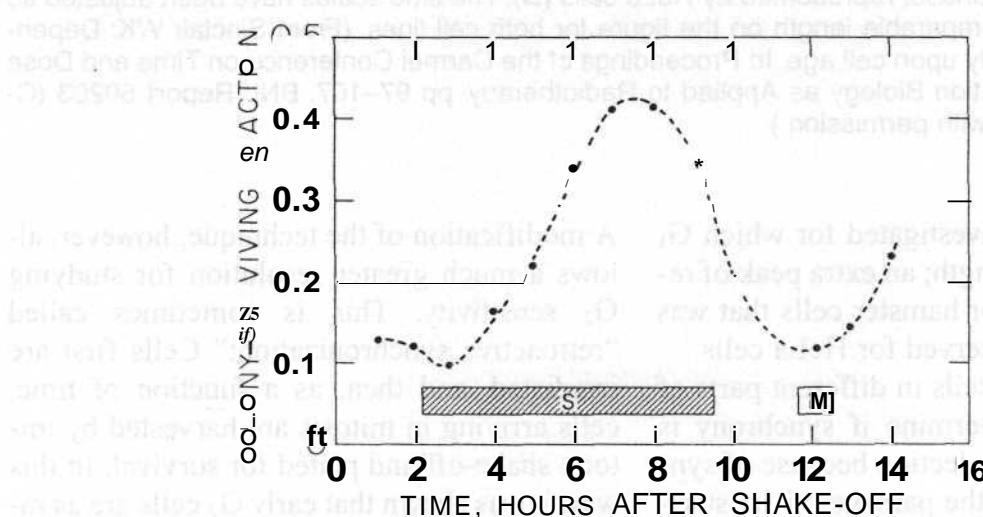
The experiments of Terasima and Tolmach with HeLa cells, in which a dose of 3 Gy (300 rad) was delivered to cultures at various intervals after mitotic harvesting of the cells, are shown in Figure 4.9. From the beginning of S phase onward, the pattern of sensitivity is very similar to that of hamster cells; the cells become progressively more resistant as they proceed toward the latter part of S, and after the cells move from S into G<sub>2</sub> their sensitivity increases rapidly as they approach mitosis. The important difference between HeLa and hamster cells is the length of the G<sub>1</sub> phase. The G<sub>1</sub> of HeLa cells is appreciably long, and there appears to be a fine structure in the age-response function during this period. At the beginning of G<sub>1</sub> there is a resistant peak, followed by a sensitive trough toward the end of G<sub>1</sub>. This pattern cannot be distinguished in the hamster cell because G<sub>1</sub> is too short.



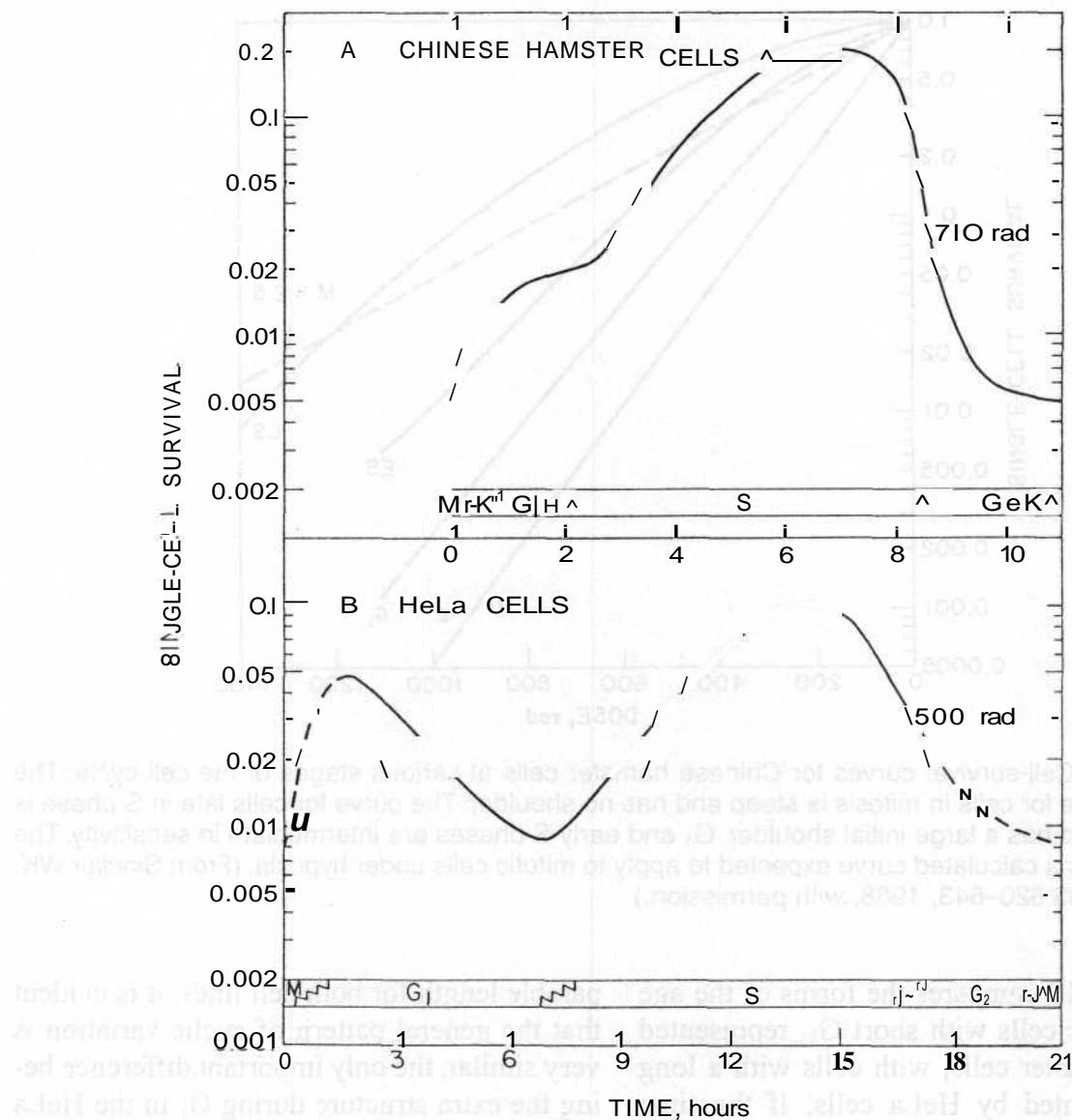
**Figure 4.8.** Cell-survival curves for Chinese hamster cells at various stages of the cell cycle. The survival curve for cells in mitosis is steep and has no shoulder. The curve for cells late in S phase is shallower and has a large initial shoulder. G<sub>i</sub> and early S phases are intermediate in sensitivity. The broken line is a calculated curve expected to apply to mitotic cells under hypoxia. (From Sinclair WK: Radiat Res 33:620-643, 1968, with permission.)

Figure 4.10 compares the forms of the age response for cells with short G<sub>i</sub>, represented by V79 hamster cells, with cells with a long d, represented by HeLa cells. If the time scales are adjusted so that S phase has a com-

parable length for both cell lines, it is evident that the general pattern of cyclic variation is very similar, the only important difference being the extra structure during G<sub>i</sub> in the HeLa cells. In later experiments, other sublines of



**Figure 4.9.** Fraction of HeLa cells surviving a dose of 3 Gy (300 rad) of x-rays administered at different times in the division cycle. Time zero represents mitosis. (Adapted from Terasima T, Tolmach LJ: Biophys J 3:11-33, 1963, with permission.)



**Figure 4.10.** Forms of age response for cells with short Gi phase, represented by hamster cells (A), and cells with long Gi phase, represented by HeLa cells (B). The time scales have been adjusted so that S phase has a comparable length on the figure for both cell lines. (From Sinclair WK: Dependence of radiosensitivity upon cell age. In Proceedings of the Carmel Conference on Time and Dose Relationships in Radiation Biology as Applied to Radiotherapy, pp 97-107. BNL Report 50203 (C-57). Upton, NY, 1969, with permission.)

hamster cells were investigated for which Gi had an appreciable length; an extra peak of resistance was noted for hamster cells that was similar to the one observed for HeLa cells.

The sensitivity of cells in different parts of G<sub>2</sub> is difficult to determine if synchrony is produced by mitotic selection because of synchrony decay during the passage of the starting population of mitotic cells through their first G<sub>1</sub> and S phases, and because G<sub>2</sub> transit times are relatively short (about 1 to 2 hours).

A modification of the technique, however, allows a much greater resolution for studying G<sub>2</sub> sensitivity. This is sometimes called "retroactive synchronization:" Cells first are irradiated, and then, as a function of time, cells arriving in mitosis are harvested by mitotic shake-off and plated for survival. In this way, it was shown that early G<sub>2</sub> cells are as radioresistant as late S cells, and late G<sub>2</sub> cells are nearly as sensitive as mitotic cells; that is, a sharp transition in radiosensitivity occurs

around the so-called x-ray transition point (now often called a "check point") for G<sub>2</sub> cell-cycle delay.

The following is a summary of the main characteristics of the variation of radiosensitivity with cell age in the mitotic cycle:

1. Cells are most sensitive at or close to mitosis.
2. Resistance is usually greatest in the latter part of S phase.
3. If G<sub>1</sub> phase has an appreciable length, a resistant period is evident early in G<sub>1</sub>, followed by a sensitive period toward the end of G<sub>1</sub>.
4. G<sub>2</sub> phase is usually sensitive, perhaps as sensitive as M phase.

A number of cell lines other than HeLa and hamster have been investigated, some of which tend to agree with these results and some of which are contradictory. The summary points listed here are widely applicable, but exceptions to every one of these generalizations have been noted for one cell line or another.

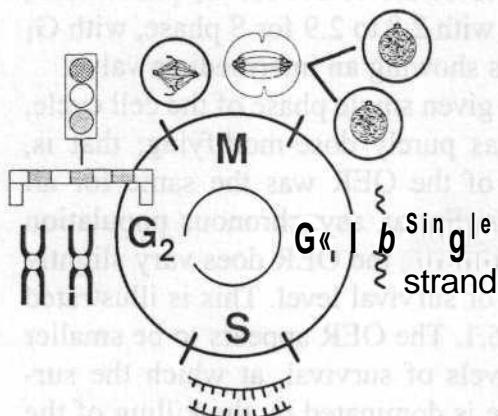
## MOLECULAR CHECKPOINT GENES

Cell-cycle progression is controlled by a family of genes known as **molecular checkpoint** genes. It has been known for many years that mammalian cells exposed to a small dose of radiation tend to experience a block in the G<sub>2</sub> phase of the cell cycle. For example, the inverse dose-rate effect reported for cells of human origin, whereby over a limited range of dose rates around 0.30 to 0.40 Gy/h ( $30^0$  rad/h) cells become more sensitive to radiation-induced cell killing as the dose rate is reduced, results from the piling up of cells in G<sub>2</sub>, which is a radiosensitive phase of the cell cycle. This is described in Chapter 5. The mechanisms for this observation in human cells are not understood in detail, but the molecular genetics in yeast have been worked out and the search is on for homology in mammalian cells.

In several strains of yeast, mutants have been isolated that are more sensitive than the

wild type both to ionizing radiation and ultraviolet light, by a factor between 10 and 100. The mutant gene has been cloned and sequenced and found to be a "molecular checkpoint gene." In the most general terms, the function of checkpoint genes is to ensure the correct order of cell-cycle events, that is, to ensure that the initiation of later events is dependent on the completion of earlier events. The particular genes involved in radiation effects halt cells in G<sub>2</sub>, so that an inventory of chromosome damage can be taken, and repair initiated and completed, before the complex task of mitosis is attempted (Fig. 4.11). Mutant cells that lose this gene function move directly into mitosis with damaged chromosomes and are therefore at a higher risk of dying, hence their greater sensitivity to radiation, or for that matter to any DNA-damaging agent.

It has been proposed that a checkpoint control monitors spindle function during mitosis. If the spindle is disrupted by a microtubular poison, progression through mitosis is



**Figure 4.11.** Diagram illustrating the site of action and function of the molecular checkpoint gene. Cells exposed to any DNA-damaging agent, including ionizing radiation, are arrested in G<sub>2</sub> phase. The function of the pause in cell-cycle progression is to allow a check of chromosome integrity before the complex task of mitosis is attempted. Cells in which the checkpoint gene is inactivated are much more sensitive to killing by  $\gamma$ -rays or ultraviolet light. The mutant gene isolated from a sensitive strain of yeast functions as a checkpoint gene.

blocked. The checkpoint control is involved in this dependency of mitosis-on spindle function. It is thought that the mechanism of action of checkpoint genes involves p34 protein kinase, levels of which control passage through mitosis. It is likely that mammalian cells that lack checkpoint genes would be sensitive not only to radiation-induced cell killing but also to carcinogenesis. Cells with damaged chromosomes that survive mitosis are likely to give rise to errors in chromosome segregation at mitosis, and this is one of the hallmarks of cancer.

#### THE EFFECT OF OXYGEN AT VARIOUS PHASES OF THE CELL CYCLE

By combining the most sophisticated techniques of flow cytometry to separate cells into different phases of the cycle with the most sensitive assays for cell survival, it has been shown that the oxygen enhancement ratio (OER) varies significantly through the cycle, at least if measured for fast-growing proliferating cells cultured *in vitro*. The OER was measured to be 2.3 to 2.4 for G<sub>2</sub> phase cells, compared with 2.8 to 2.9 for S phase, with G<sub>1</sub> phase cells showing an intermediate value.

For any given single phase of the cell cycle, oxygen was purely dose-modifying; that is, the value of the OER was the same for all dose levels. For an asynchronous population of cells, however, the OER does vary slightly with dose or survival level. This is illustrated in Figure 6.1. The OER appears to be smaller at high levels of survival, at which the survival curve is dominated by the killing of the most sensitive moieties of the population; the OER appears to be larger at higher doses and lower levels of survival, at which the response of the most resistant (S-phase) cells, which also happen to exhibit the largest OER, dominates.

This is an interesting radiobiologic observation, but the small change of OER is of little or no clinical significance in radiation therapy.

#### THE AGE-RESPONSE FUNCTION FOR A TISSUE *IN VIVO*

Most studies of the variation in radiosensitivity with phase of the mitotic cycle have been done with mammalian cells cultured *in vitro* because of the ease with which they can be made to divide synchronously. The mitotic harvest technique is clearly only applicable to monolayer cultures, but techniques that involve a drug, such as hydroxyurea, to produce a synchronously dividing population can be applied to some organized tissues.

The crypt cells in the mouse jejunum are a classic self-renewal tissue. (The technique used to obtain a survival curve for these cells was described in Chapter 3.) The rapidly dividing crypt cells can be synchronized by giving each mouse five intraperitoneal injections of hydroxyurea every hour. The rationale for this regimen is that all S cells are killed by the drug, and cells in other phases of the cycle are accumulated at the G<sub>1</sub>-S boundary for at least 4 hours (the overall time of the five injections). Figure 4.12, from Withers and his colleagues, shows the response of the jejunal crypt cells to a single dose of 11 Gy (1100 rad) of  $\gamma$ -rays (uppermost curve) delivered at various times after the synchronizing action of the five injections of hydroxyurea. The number of crypt cells per circumference of the sectioned jejunum varies by a factor of 100, according to the phase in the cycle at which the radiation is delivered, ranging from about two survivors per circumference for irradiation 2 hours after the last injection of hydroxyurea to about 200 survivors per circumference by 6 hours. The DNA synthetic activity of the synchronized jejunal mucosa was monitored by injecting groups of mice with tritiated thymidine at hourly intervals after the last injection of hydroxyurea and subsequently removing a sample of the jejunum and assaying the radioactive content. The bottom curve of Figure 4.12 shows the variation of thymidine uptake with time. The first wave of the thymidine uptake represents the period of DNA synthesis of the synchronized crypt cells. The peak coincides closely with the pe-

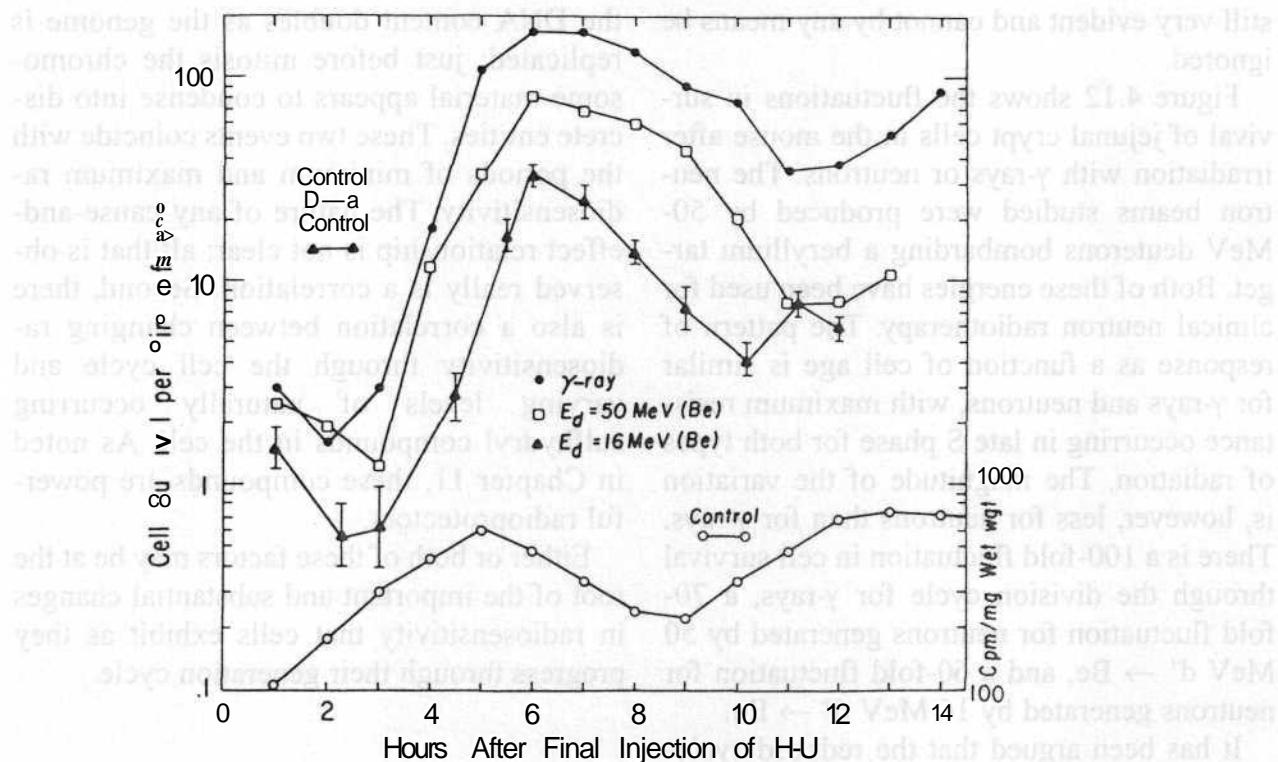


Figure 4.12. The upper three curves represent fluctuations in the survival of jejunal crypt cells exposed to  $\gamma$ -rays or neutrons as they pass through the cell cycle after synchronization with hydroxyurea (H-U). The doses were 11 Gy (1100 rad) of  $\gamma$ -rays; 7 Gy (700 rad) of neutrons generated by 50 MeV  $d^- \rightarrow$  Be; and 6 Gy (600 rad) of neutrons generated by 16 MeV  $d^- \rightarrow$  Be. The lower curve represents the uptake of tritiated thymidine (expressed as counts per minute) per wet weight of jejunum as a function of time after the last injection of hydroxyurea. The first wave indicates crypt stem cells passing through S phase after synchronization at GrS phase by hydroxyurea. (From Withers HR, Mason K, Reid BO, et al.: Response of mouse intestine to neutrons and gamma rays in relation to dose fractionation and division cycle. Cancer 34:39-47, 1974, with permission.)

nod of maximum resistance to x-rays (about 5 hours after the last injection of hydroxyurea).

These data indicate clearly that the radiosensitivity of crypt cells in the mouse jejunum varies substantially with the phase of the cell cycle at which the radiation is delivered. Further, the pattern of response in this organized normal tissue, with a sensitive period between Gi and S and maximum radioreistance late in S, is very similar to that characteristic of many cell lines cultured *in vitro*.

#### VARIATION OF SENSITIVITY WITH CELL AGE FOR NEUTRONS

With the introduction of neutrons for use in radiotherapy in place of conventional modalities,

such as x- or  $\gamma$ -rays, all possible radiobiologic parameters of the two types of radiation were compared.

It was found that the variation in radiosensitivity as a function of cell age was qualitatively similar for neutrons and x-rays; that is, with both types of radiation, maximum sensitivity is noted at or close to mitosis, and maximum resistance is evident late in S phase. There are, however, quantitative differences between neutrons and x-rays in this respect, as in every other. The range of radiosensitivity between the most resistant and the most sensitive phases of the cell cycle is much less for fast neutrons than for x-rays. Although the variation of sensitivity with cell age is reduced in neutrons compared with x-rays, it is

still very evident and cannot by any means be ignored.

Figure 4.12 shows the fluctuations in survival of jejunal crypt cells in the mouse after irradiation with  $\gamma$ -rays or neutrons. The neutron beams studied were produced by 50-MeV deuterons bombarding a beryllium target. Both of these energies have been used for clinical neutron radiotherapy. The pattern of response as a function of cell age is similar for  $\gamma$ -rays and neutrons, with maximum resistance occurring in late S phase for both types of radiation. The magnitude of the variation is, however, less for neutrons than for  $\gamma$ -rays. There is a 100-fold fluctuation in cell survival through the division cycle for  $\gamma$ -rays, a 70-fold fluctuation for neutrons generated by 50 MeV  $d^+ \rightarrow Be$ , and a 60-fold fluctuation for neutrons generated by 16 MeV  $d^+ \rightarrow Be$ .

It has been argued that the reduced cycle-related fluctuations in radiation response that occur with neutrons could represent an advantage over conventional therapeutic radiation modalities, such as x- or  $\gamma$ -rays. There could be an important difference in the response of normal tissues and of neoplastic tissues to neutrons if the age-density distributions of normal and neoplastic tissues were dissimilar as a consequence of different rates of proliferation. At all events the reduced age-response function seen with neutrons represents a difference between this relatively densely ionizing radiation and x-rays over and above the oxygen effect.

### MECHANISMS FOR THE AGE-RESPONSE FUNCTION

The reasons for the sensitivity changes through the cell cycle are not at all understood. Several correlations have been proposed, of which two are mentioned here. First, if DNA is the primary target for radiation-induced cell lethality, as commonly is supposed, then changes in the amount or form of the DNA might be expected to result in variations in sensitivity. During S phase

the DNA content doubles as the genome is replicated; just before mitosis the chromosome material appears to condense into discrete entities. These two events coincide with the periods of minimum and maximum radiosensitivity. The nature of any cause-and-effect relationship is not clear; all that is observed really is a correlation. Second, there is also a correlation between changing radiosensitivity through the cell cycle and varying levels of naturally occurring sulphydryl compounds in the cell. As noted in Chapter 11, these compounds are powerful radioprotectors.

Either or both of these factors may be at the root of the important and substantial changes in radiosensitivity that cells exhibit as they progress through their generation cycle.

### THE POSSIBLE IMPLICATIONS OF THE AGE-RESPONSE FUNCTION IN RADIOTHERAPY

If a single dose of radiation is delivered to a population of cells that are asynchronous—that is, distributed throughout the cell cycle—the effect is different on cells occupying different phases of the cell cycle at the time of the radiation exposure. A greater proportion of cells is killed in the sensitive portions of the cell cycle, such as those at or close to mitosis; a smaller proportion of those in the DNA synthetic phase is killed. The overall effect is that a dose of radiation, to some extent, tends to synchronize the cell population, leaving the majority of cells in a resistant phase of the cycle. Between dose fractions, movement of cells through the cycle into more sensitive phases may be an important factor in "sensitizing" a cycling population of tumor cells to later doses in fractionated regimen. This is considered sensitization resulting from reassortment. It results in a therapeutic gain, because sensitization by this mechanism occurs only in rapidly dividing cells and not in late-responding normal tissues.

### SUMMARY OF PERTINENT CONCLUSIONS

- The cell cycle for mammalian cells can be divided into four phases: mitosis (M), followed by G<sub>1</sub>, followed by the DNA synthetic phase (S), then G<sub>2</sub>, and into mitosis again.
- The phases of the cycle are regulated by the periodic activation of different members of the cyclin-dependent kinase family.
- The fastest cycling mammalian cells in culture, and crypt cells in the intestinal epithelium, have cycle times as short as 9 to 10 hours. Stem cells in resting mouse skin may have cycle times of more than 200 hours. Most of this difference results from the varying length of G<sub>1</sub>, the most variable phase of the cycle. M and S phases do not vary much.
- In general, cells are most radiosensitive in M and G<sub>2</sub> phases and most resistant in late S phase.
- For cells with longer cell-cycle times and significantly long G<sub>1</sub> phases, there is a second peak of resistance early in G<sub>1</sub>.
- Molecular checkpoint genes stop cells in G<sub>2</sub> if exposed to x-rays or any other DNA-damaging agent, to allow the chromosomes to be checked for integrity before the complex task of mitosis is attempted.
- The oxygen enhancement ratio varies little with phase of the cell cycle but may be slightly lower for cells in G<sub>1</sub> than for cells in S.
- The age-response function for crypt cells in the mouse jejunum is similar to that for cells in culture. This is the only tissue that has been studied.
- The age-response function for neutrons is qualitatively similar to that for x-rays, but the magnitude of changes through the cycle is smaller.
- The pattern of resistance and sensitivity correlates with the level of sulphydryl compounds in the cell. Sulphydryls are natural radioprotectors and tend to be at their highest levels in S phase and at their lowest near mitosis.
- Variations in sensitivity through the cell cycle may be important in radiation therapy because they lead to "sensitization resulting from reassortment" in a fractionated regimen.

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# 5

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## Repair of Radiation Damage and the Dose-Rate Effect

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CLASSIFICATION OF RADIATION DAMAGE  
POTENTIALLY LETHAL DAMAGE  
SUBLETHAL DAMAGE REPAIR  
MECHANISM OF SUBLETHAL DAMAGE  
REPAIR  
REPAIR AND RADIATION QUALITY  
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SUMMARY OF PERTINENT CONCLUSIONS

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### CLASSIFICATION OF RADIATION DAMAGE

Radiation damage to mammalian cells can be divided into three categories: (1) **lethal damage**, which is irreversible and irreparable and, by definition, leads irrevocably to cell death; (2) **sublethal damage**, which under normal circumstances can be repaired in hours unless additional sublethal damage is added (*e.g.*, from a second dose of radiation) with which it can interact to form lethal damage (sublethal damage repair, therefore, is manifest by the increase in survival observed if a dose of radiation is split into two fractions separated by a time interval); and (3) **potentially lethal damage** (PLD), the component of radiation damage that can be modified by postirradiation environmental conditions. All three are simply operational terms, because in mammalian cells the mechanisms of repair and radioresistance are not fully understood at the molecular level.

In several species of yeast, mutants have been isolated that are very sensitive to killing by x-rays (or by ultraviolet light). Many of the wild-type versions of the defective genes have been isolated, and in some cases their DNA sequences have been determined. The gene products appear either to be involved directly in the repair process or to function as molecular checkpoint controlling elements. (Molecular checkpoint genes are described in Chapter 16.)

In mammalian cells, the first repair gene to be isolated was associated with correcting DNA damage produced by mitomycin C. This gene is located on human chromosome 18, and it has been characterized and its DNA sequence determined. The situation is much more difficult in the case of x-rays, because mutant mammalian cell lines that are very radiosensitive compared with the wild type are simply not available. For example, in the case of mitomycin C, the mutant and wild-type cells differed in sensitivity to the drug by a

factor of about 500, whereas the most radiosensitive mammalian mutants differ from normal cells by a factor of only 2 or 3. This makes the isolation of normal x-ray repair genes technically difficult. A human gene capable of correcting the x-ray sensitivity of a mutant Chinese hamster cell line, however, has been isolated and localized to chromosome 19, and its DNA sequence also has been ascertained. The gene in the human genetic disorder ataxia telangiectasia, which is associated with sensitivity to x-ray cell killing, as

well as a predisposition to cancer, has been cloned and sequenced.

### POTENTIALLY LETHAL DAMAGE

Varying environmental conditions after exposure to x-rays can influence the proportion of cells that survive a given dose because of the expression or repair of PLD. This damage is potentially lethal because under ordinary circumstances it causes cell death, but if survival is increased as a result

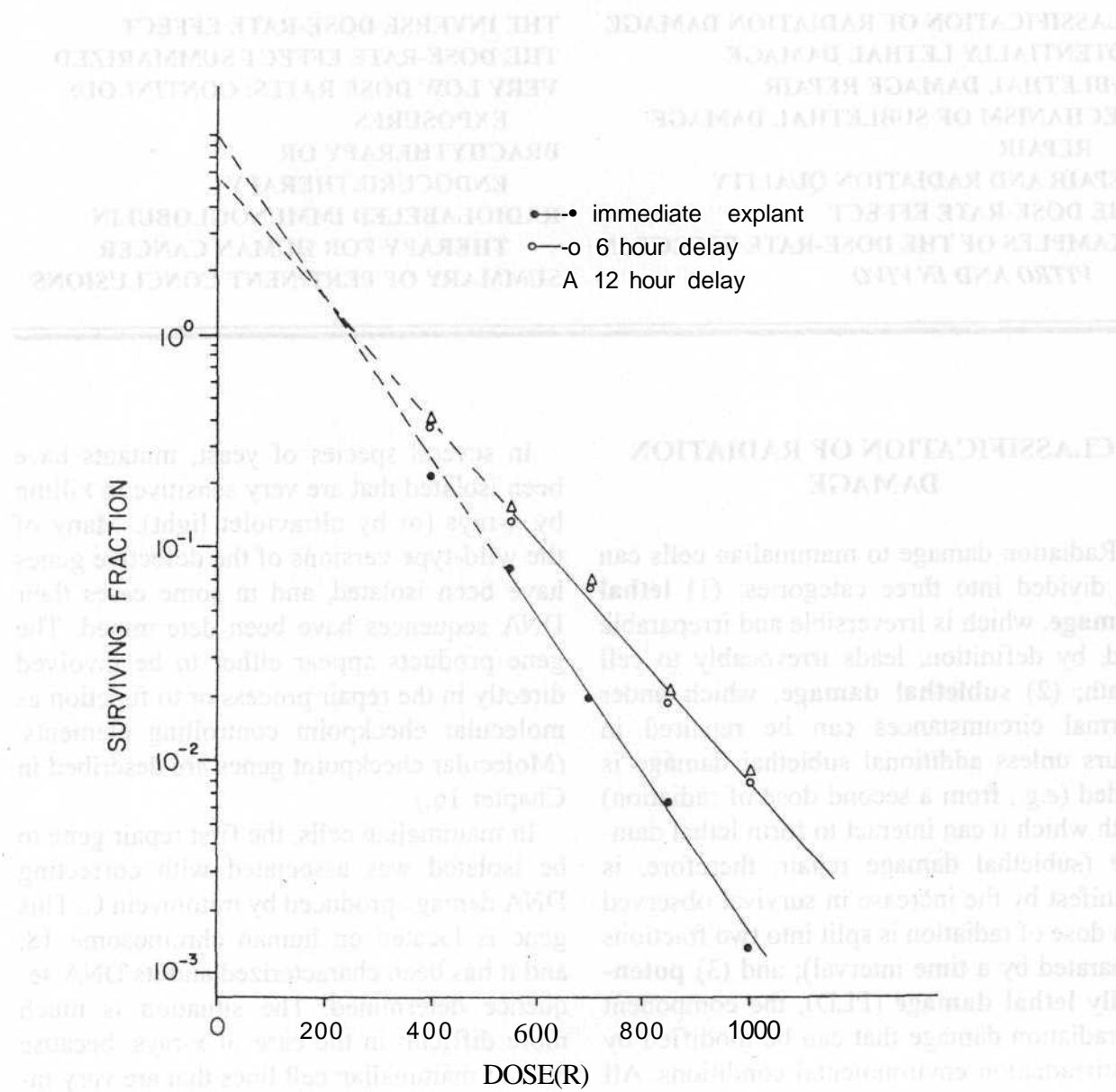


Figure 5.1. X-ray survival curves for density-inhibited stationary-phase cells, subcultured (trypsinized and plated) either immediately or 6 to 12 hours after irradiation. Cell survival is enhanced if cells are left in the stationary phase after irradiation, allowing time for the repair of potentially lethal damage. (From Little JB, Hahn GM, Frindel E, Tubiana M: Repair of potentially lethal radiation damage in vitro and in vivo. Radiology 106:689-694, 1973, with permission.)

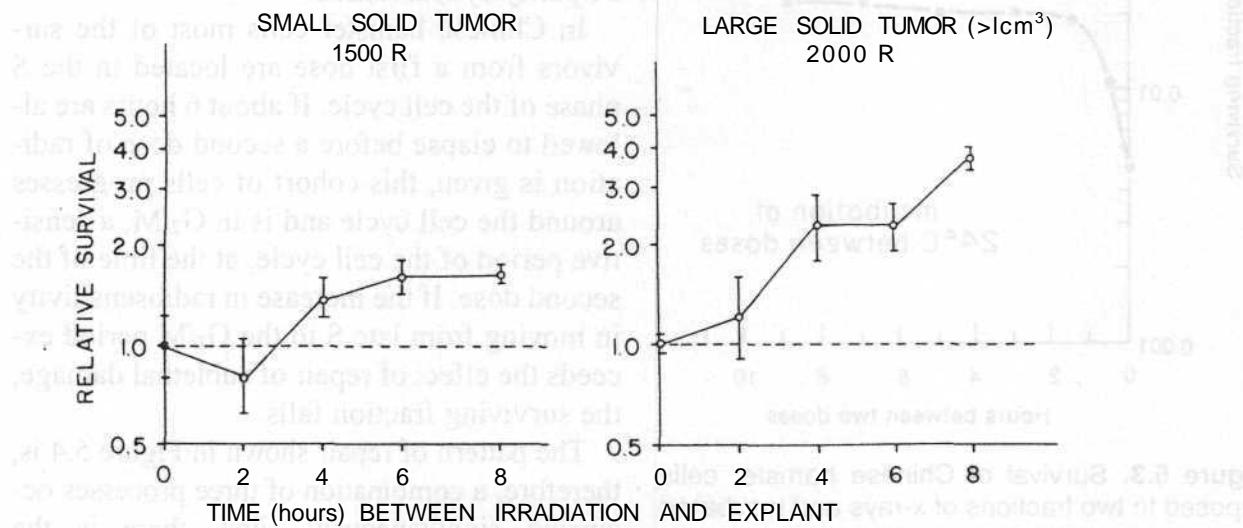
of the manipulation of the postirradiation environment, PLD is considered to have been repaired. PLD is repaired if cells are incubated in balanced salt solution instead of full growth medium for several hours after irradiation. This is a drastic treatment, however, and does not mimic a physiologic condition that is ever likely to occur. Little and his colleagues chose to study PLD repair in density-inhibited stationary-phase cell cultures, which are considered to be a better *in vitro* model for tumor cells *in vivo* (Fig. 5.1). Cell survival was enhanced considerably if the cells were allowed to remain in the density-inhibited state for 6 to 12 hours after irradiation before being subcultured and assayed for colony-forming ability.

The relevance of PLD to radiotherapy became much more obvious when it was shown that repair, comparable in magnitude and kinetics to that found *in vitro*, also occurred *in vivo* in experimental tumors. In this case, repair took the form of significantly enhanced cell survival if several hours were allowed to elapse between irradiation of the tumor *in situ*

and removal of the cells from the host to assess their reproductive integrity (Fig. 5.2).

To summarize the available experimental data, there is general agreement that PLD is repaired and the fraction of cells surviving a given dose of x-rays is enhanced if postirradiation conditions are suboptimal for growth, so that cells do not have to attempt the complex process of mitosis while their chromosomes are damaged. If mitosis is delayed by suboptimal growth conditions, DNA damage can be repaired.

The importance of PLD repair to clinical radiotherapy is a matter of debate. That it occurs in transplantable animal tumors has been documented beyond question, and there is no reason to suppose that it does not occur in human tumors. It has been suggested that the radioresistance of certain types of human tumors is linked to their ability to repair PLD; that is, radiosensitive tumors repair PLD inefficiently, but radioresistant tumors have efficient mechanisms to repair PLD. This is an attractive hypothesis, but it has not stood the test of time.



**Figure 5.2.** Repair of potentially lethal damage in mouse fibrosarcomas. The tumors were irradiated *in situ* and then removed and prepared into single-cell suspensions. The number of survivors was determined by their ability to form colonies *in vitro*. The fraction of cells surviving a given dose increases if a time interval is allowed between irradiation and removal of the tumor, because during this interval potentially lethal damage is repaired. (From Little JB, Hahn GM, Frindel E, Tubiana M: Repair of potentially lethal radiation damage *in vitro* and *in vivo*. Radiology 106:689-694, 1973, with permission.)

### SUBLETHAL DAMAGE REPAIR

**Sublethal damage repair** is the operational term for the increase in cell survival that is observed if a given radiation dose is split into two fractions separated by a time interval.

Figure 5.3 shows data obtained in a split-dose experiment with cultured Chinese hamster cells. A single dose of 15.58 Gy (1,558 rad) leads to a surviving fraction of 0.005. If the dose is divided into two equal fractions, separated by 30 minutes, the surviving fraction is already appreciably higher than for a single dose. As the time interval is extended, the surviving fraction increases until a plateau is reached at about 2 hours, corresponding to a surviving fraction of 0.02. This represents about four times as many surviving cells as for the dose given in a single exposure. A further increase in the time interval between the dose fractions is not accompanied by any additional increment in survival. The increase in

survival in a split-dose experiment results from the repair of sublethal radiation damage.

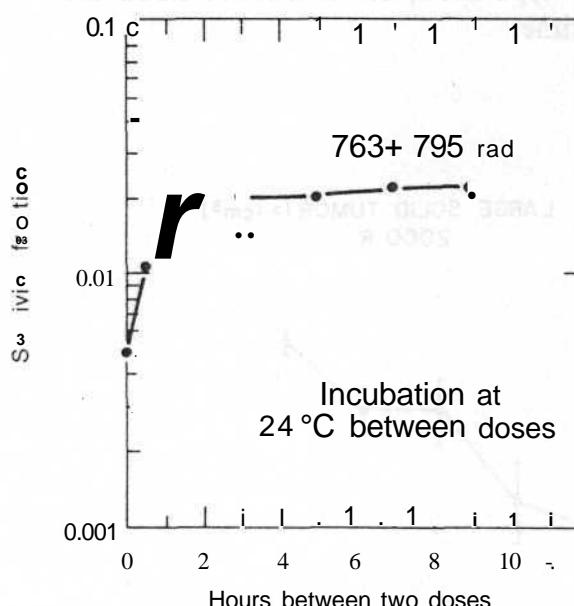
The data shown in Figure 5.3 were obtained with cultured mammalian cells maintained at room temperature ( $24^{\circ}\text{C}$ ) between the dose fractions to prevent the cells from moving through the cell cycle during this interval. This rather special experiment is described first because it illustrates the phenomenon of the repair of sublethal radiation damage, uncomplicated by the movement of cells through the cell cycle.

Figure 5.4 shows the results of a parallel experiment in which cells were exposed to split doses and maintained at their normal growing temperature of  $37^{\circ}\text{C}$ . The pattern of repair seen in this case differs from that observed for cells kept at room temperature. In the first few hours prompt repair of sublethal damage is again evident, but at longer intervals between the two split doses the surviving fraction of cells decreases again.

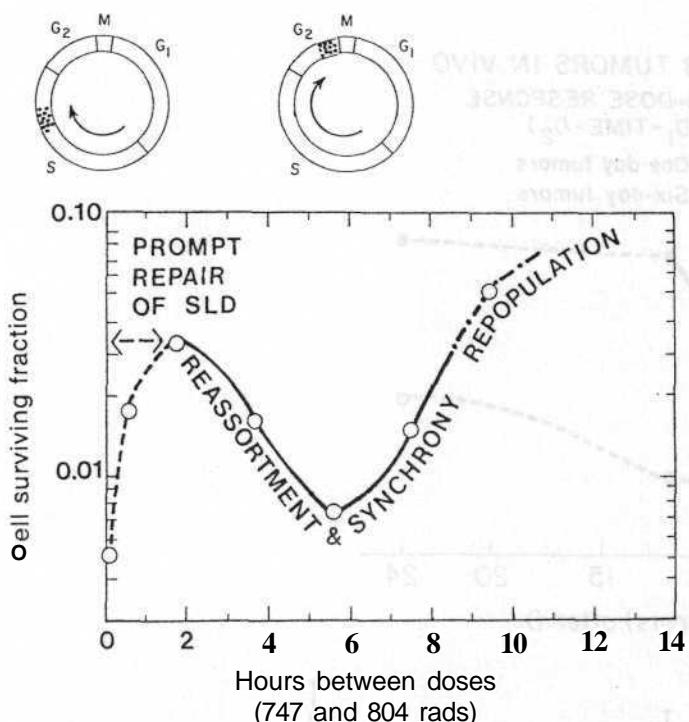
An understanding of this phenomenon is based on the age-response function described in Chapter 4. If an asynchronous population of cells is exposed to a large dose of radiation, more cells are killed in the sensitive than in the resistant phases of the cell cycle. The surviving population of cells, therefore, tends to be partly synchronized.

In Chinese hamster cells most of the survivors from a first dose are located in the S phase of the cell cycle. If about 6 hours are allowed to elapse before a second dose of radiation is given, this cohort of cells progresses around the cell cycle and is in G<sub>2</sub>/M, a sensitive period of the cell cycle, at the time of the second dose. If the increase in radiosensitivity in moving from late S to the G<sub>2</sub>/M period exceeds the effect of repair of sublethal damage, the surviving fraction falls.

The pattern of repair shown in Figure 5.4 is, therefore, a combination of three processes occurring simultaneously. First, there is the prompt repair of sublethal radiation damage. Second, there is progression of cells through the cell cycle during the interval between the split doses, which has been termed **reassortment**. Third, there is an increase of surviving fraction resulting from cell division, or **repopulation**, if



**Figure 5.3.** Survival of Chinese hamster cells exposed to two fractions of x-rays and incubated at room temperature for various time intervals between the two exposures. (From Elkind MM, Sutton-Gilbert H, Moses WB, Alescio T, Swain RB: Radiation response of mammalian cells in culture: V. Temperature dependence of the repair of x-ray damage in surviving cells (aerobic and hypoxic). (Radiat Res 25:359-376, 1965, with permission.)



**Figure 5.4.** Survival of Chinese hamster cells exposed to two fractions of x-rays and incubated at 37°C for various time intervals between the two doses. The survivors of the first dose are predominantly in a resistant phase of the cycle (late S). If the interval between doses is about 6 hours, these resistant cells have moved to the G<sub>2</sub>-M phase, which is sensitive. (Adapted from Elkind MM, Sutton-Gilbert H, Moses WB, Alescio T, Swain RB: Radiation response of mammalian cells in culture: V. Temperature dependence of the repair of x-ray damage in surviving cells (aerobic and hypoxic). Radiat Res 25:359-376, 1965, with permission.)

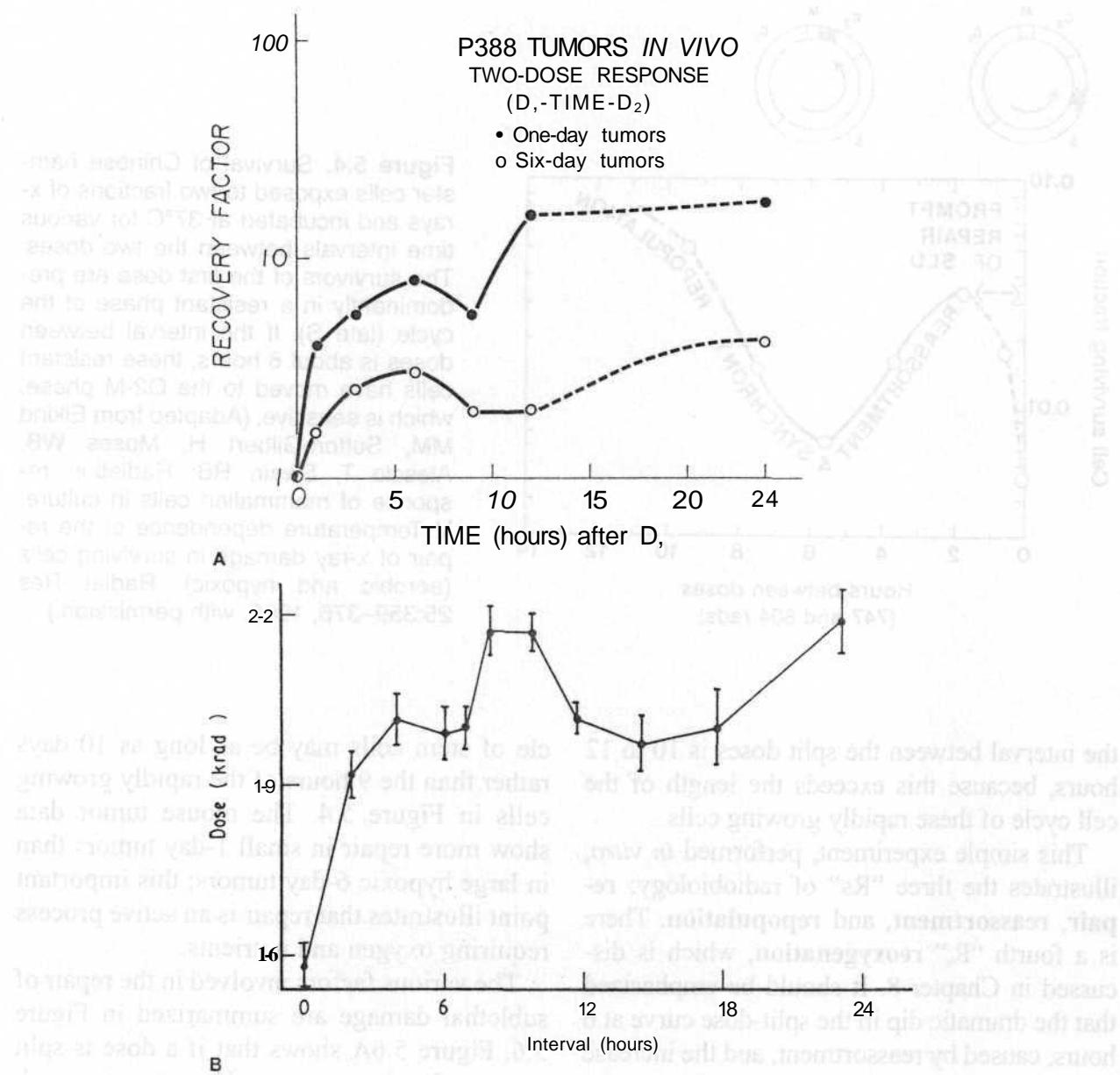
the interval between the split doses is 10 to 12 hours, because this exceeds the length of the cell cycle of these rapidly growing cells.

This simple experiment, performed *in vitro*, illustrates the three "Rs" of radiobiology: **repair**, **reassortment**, and **repopulation**. There is a fourth "R," **reoxygenation**, which is discussed in Chapter 8. It should be emphasized that the dramatic dip in the split-dose curve at 6 hours, caused by reassortment, and the increase in survival by 12 hours, because of repopulation, are seen only for rapidly growing cells. Hamster cells in culture have a cycle time of only 9 or 10 hours. The time sequence of these events would be longer in more slowly proliferating normal tissues *in vivo*.

Repair of sublethal radiation damage has been demonstrated in just about every biologic test system for which a quantitative endpoint is available. Figure 5.5 illustrates the pattern for repair of sublethal radiation damage in two *in vivo* systems in mice, P388 lymphocytic leukemia and skin cells. In neither case is there a dramatic dip in the curve at 6 hours resulting from movement of cells through the cycle, because the cell cycle is long. In resting skin, for example, the cell cy-

cle of stem cells may be as long as 10 days rather than the 9 hours of the rapidly growing cells in Figure 5.4. The mouse tumor data show more repair in small 1-day tumors than in large hypoxic 6-day tumors; this important point illustrates that repair is an active process requiring oxygen and nutrients.

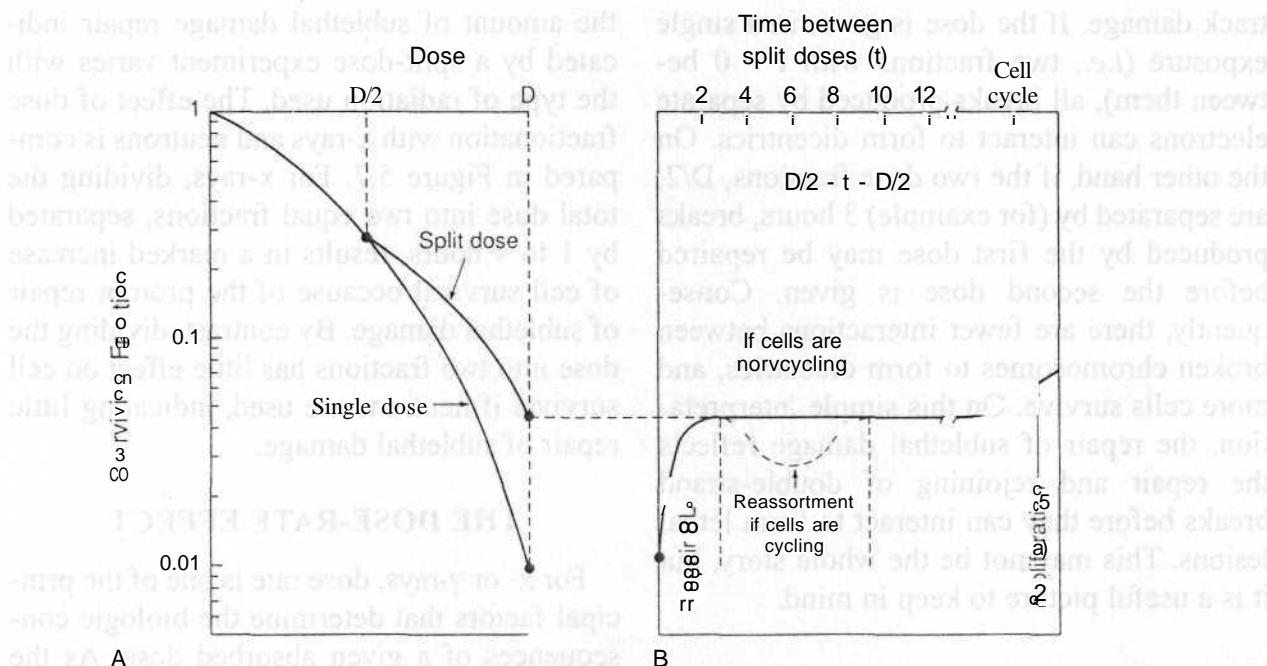
The various factors involved in the repair of sublethal damage are summarized in Figure 5.6. Figure 5.6A shows that if a dose is split into two fractions separated by a time interval, more cells survive than for the same total dose given in a single fraction, because the shoulder of the curve must be repeated with each fraction. In general, there is a good correlation between the extent of repair of sublethal damage and the size of the shoulder of the survival curve. This is not surprising, because both are manifestations of the same basic phenomenon: the accumulation and repair of sublethal damage. Some mammalian cells are characterized by a survival curve with a broad shoulder, and split-dose experiments then indicate a substantial amount of sublethal damage repair. Other types of cells show survival curves with minimal shoulders, and this is reflected in more limited repair of sublethal



**Figure 5.5.** Repair of sublethal damage in two *in vivo* mammalian cell systems. A: Split-dose experiments with P388 lymphocytic leukemia cells in the mouse. The recovery factor is the ratio of the surviving fraction resulting from two-dose fractionation to the survival from a single equivalent dose. One-day-old tumors are composed predominantly of oxygenated cells; the cells in 6-day-old tumors are hypoxic. (From Belli JA, Dicus GJ, Bonte FJ: Radiation response of mammalian tumor cells: 1. Repair of sublethal damage *in vivo*. *J Natl Cancer Inst* 38:673-682, 1967, with permission.) B: Split-dose experiments with skin epithelial cells in the mouse. The total x-ray dose, given as two fractions, required to result in one surviving epithelial cell per square millimeter is plotted against the time interval between the two doses. (From Emery EW, Denekamp J, Ball MM: *Radiat Res* 41:450, 1970, with permission.)

damage. In the terminology of the linear-quadratic ( $a/[3]$ ) description of the survival curve, it is the quadratic component (3) that causes the curve to bend and results in the sparing effect of a split dose. A large shoulder corresponds to a small  $a/p$  ratio.

The time course of the increase in cell survival that results from the repair of sublethal damage is charted in Figure 5.6B. As the time interval between the two dose fractions is increased, there is a rapid increase in the fraction of cells surviving, owing to the prompt



**Figure 5.6.** Summary of the repair of sublethal damage as evidenced by a split-dose experiment. A: If the dose is delivered in two fractions separated by a time interval, there is an increase in cell survival because the shoulder of the curve must be expressed each time. B: The fraction of cells surviving a split dose increases as the time interval between the two dose fractions increases. As the time interval increases from 0 to 2 hours, the increase in survival results from the repair of sublethal damage. In cells with a long cell cycle, or that are out of cycle, there is no further increase in cell survival by separating the dose by more than 2 or 3 hours. In a rapidly dividing cell population, there is a dip in cell survival caused by reassortment. However, as shown in Figure 5.5, if the time interval between the split doses exceeds the cell cycle, there is an increase in cell survival owing to proliferation or repopulation between the doses.

repair of sublethal damage. This repair is complete by 1 or 2 hours for cells in culture but may take longer for late-responding tissues *in vivo* (Chapter 22). As the time interval between the two dose fractions is increased, there is a dip in the curve owing to the movement of surviving cells through the cell cycle, as explained in Figure 5.4. This occurs only in a population of fast-cycling cells. In cells that are noncycling there can be no dip. If the time interval between the two dose fractions exceeds the cell cycle, there is an increase in the number of cells surviving, because of cell proliferation; that is, cells can double in number between the dose fractions.

### MECHANISM OF SUBLETHAL DAMAGE REPAIR

In Chapter 3, evidence was summarized of the correlation between cell killing and the

production of asymmetric chromosomal aberrations, such as dicentrics and rings. This in turn is a consequence of an interaction between two (or more) double-strand breaks in the DNA. On this interpretation the repair of sublethal damage is simply the repair of double-strand breaks. If a dose is split into two parts separated by a time interval, some of the double-strand breaks produced by the first dose are rejoined and repaired before the second dose. The breaks in two chromosomes that must interact to form a lethal lesion such as a dicentric may be formed by (1) a single track breaking both chromosomes (*i.e.*, single-track damage) or (2) separate tracks breaking the two chromosomes (*i.e.*, multiple-track damage).

The component of cell killing that results from single-track damage is the same whether the dose is given in a single exposure or fractionated. The same is not true of multiple-

track damage. If the dose is given in a single exposure (*i.e.*, two fractions with  $t = 0$  between them), all breaks produced by separate electrons can interact to form dicentrics. On the other hand, if the two dose fractions,  $D/2$ , are separated by (for example) 3 hours, breaks produced by the first dose may be repaired before the second dose is given. Consequently, there are fewer interactions between broken chromosomes to form dicentrics, and more cells survive. On this simple interpretation, the repair of sublethal damage reflects the repair and rejoining of double-strand breaks before they can interact to form lethal lesions. This may not be the whole story, but it is a useful picture to keep in mind.

### REPAIR AND RADIATION QUALITY

For a given biologic test system, the shoulder on the acute survival curve and, therefore,

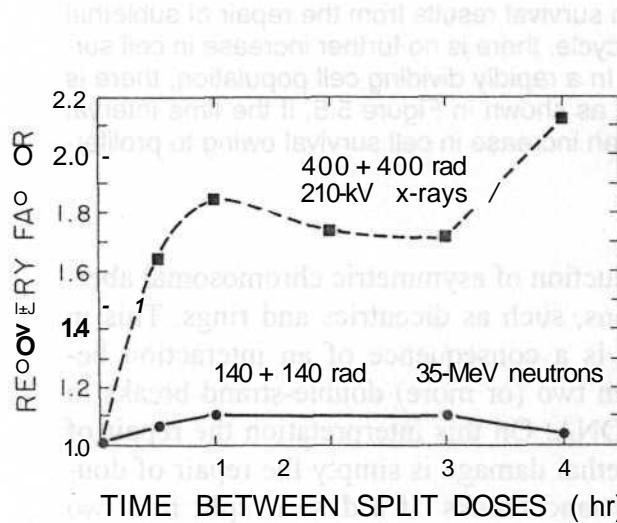


Figure 5.7. Split-dose experiments with Chinese hamster cells. For 210-kV x-rays, two 4-Gy (400-rad) doses, separated by a variable interval, were compared with a single dose of 8 Gy (800 rad). For neutrons (35-MeV  $d^+ \rightarrow Be$ ), two 1.4-Gy (140-rad) doses were compared with a single exposure of 2.8 Gy (280 rad). The data are plotted in terms of the recovery factor, defined as the ratio of surviving fractions for a given dose delivered as two fractions compared with a single exposure. It is evident that repair of sublethal damage during the interval between split doses is virtually nonexistent for neutrons but is a significant factor for x-rays. (From Hall EJ, Roizin-Towle L, Theus RB, August RS: Radiology 117:173-178, 1975, with permission.)

the amount of sublethal damage repair indicated by a split-dose experiment varies with the type of radiation used. The effect of dose fractionation with x-rays and neutrons is compared in Figure 5.7. For x-rays, dividing the total dose into two equal fractions, separated by 1 to 4 hours, results in a marked increase of cell survival because of the prompt repair of sublethal damage. By contrast, dividing the dose into two fractions has little effect on cell survival if neutrons are used, indicating little repair of sublethal damage.

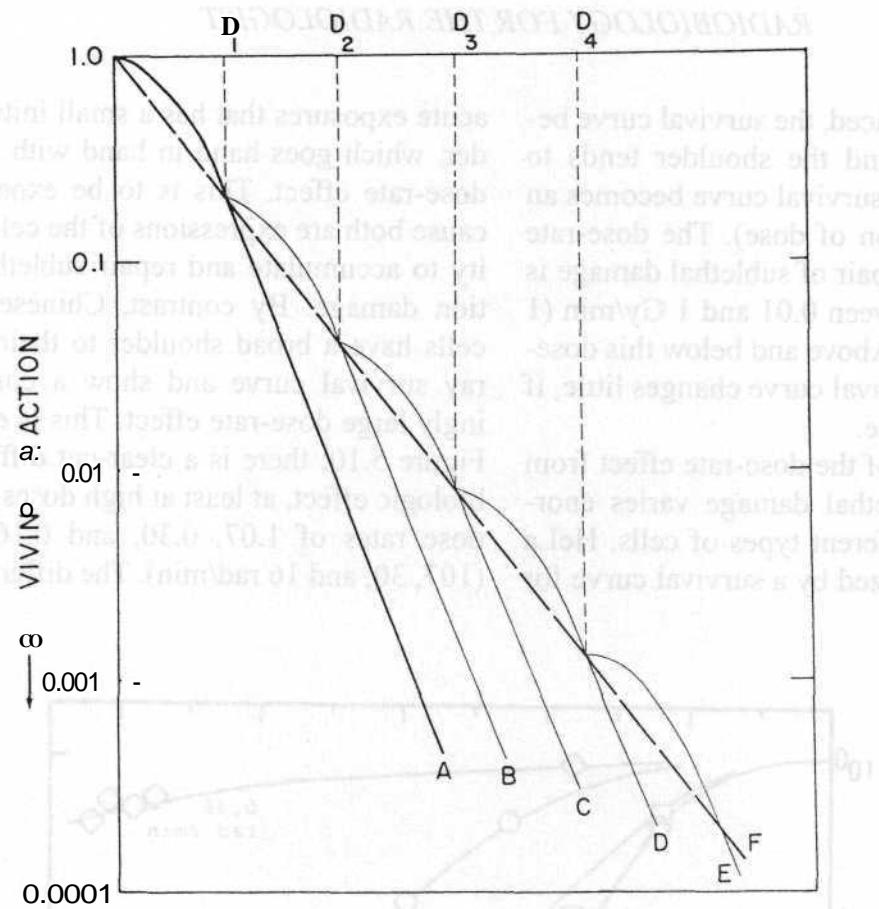
### THE DOSE-RATE EFFECT

For x- or y-rays, dose rate is one of the principal factors that determine the biologic consequences of a given absorbed dose. As the dose rate is lowered and the exposure time extended, the biologic effect of a given dose generally is reduced.

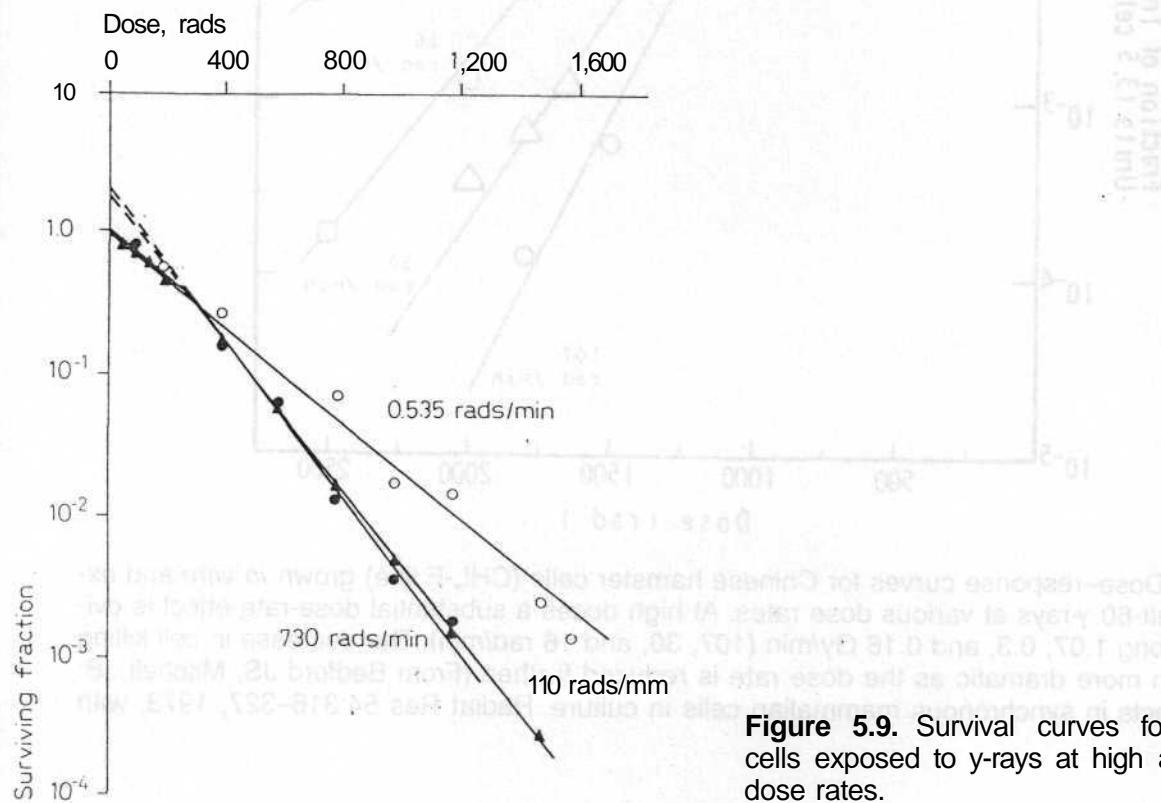
The classic dose-rate effect, which is very important in radiotherapy, results from the repair of sublethal damage that occurs during a long radiation exposure. To illustrate this principle, Figure 5.8 shows an idealized experiment in which each dose ( $D_2$ ,  $D_j$ ,  $D_4$ , and so on) is delivered in a number of equal fractions of size  $D$ , with a time interval between fractions that is sufficient for repair of sublethal damage to be complete. The shoulder of the survival curve is repeated with each fraction. The broken line,  $F$ , shows the overall survival curve that would be observed if only single points were determined, corresponding to equal dose increments. This survival curve has no shoulder. Because continuous low dose-rate irradiation may be considered to be an infinite number of infinitely small fractions, the survival curve under these conditions also would be expected to have no shoulder and to be shallower than for single acute exposures.

### EXAMPLES OF THE DOSE-RATE EFFECT IN VITRO AND IN VIVO

Survival curves for HeLa cells cultured *in vitro* over a wide range of dose rates, from 7.3 Gy/min to 0.535 cGy/mm (730 to 0.535 rad/min), are summarized in Figure 5.9. As



**Figure 5.8.** Idealized fractionation experiment. Curve A is the survival curve for single acute exposures of x-rays. Curve F is obtained if each dose is given as a series of small fractions of size  $D_i$  with an interval between fractions sufficient for repair of sublethal damage to take place. Multiple small fractions approximate to a continuous exposure to a low dose rate. (From Elkind MM, Whitmore GF: Radiobiology of Cultured Mammalian Cells. New York, Gordon and Breach, 1967, with permission.)

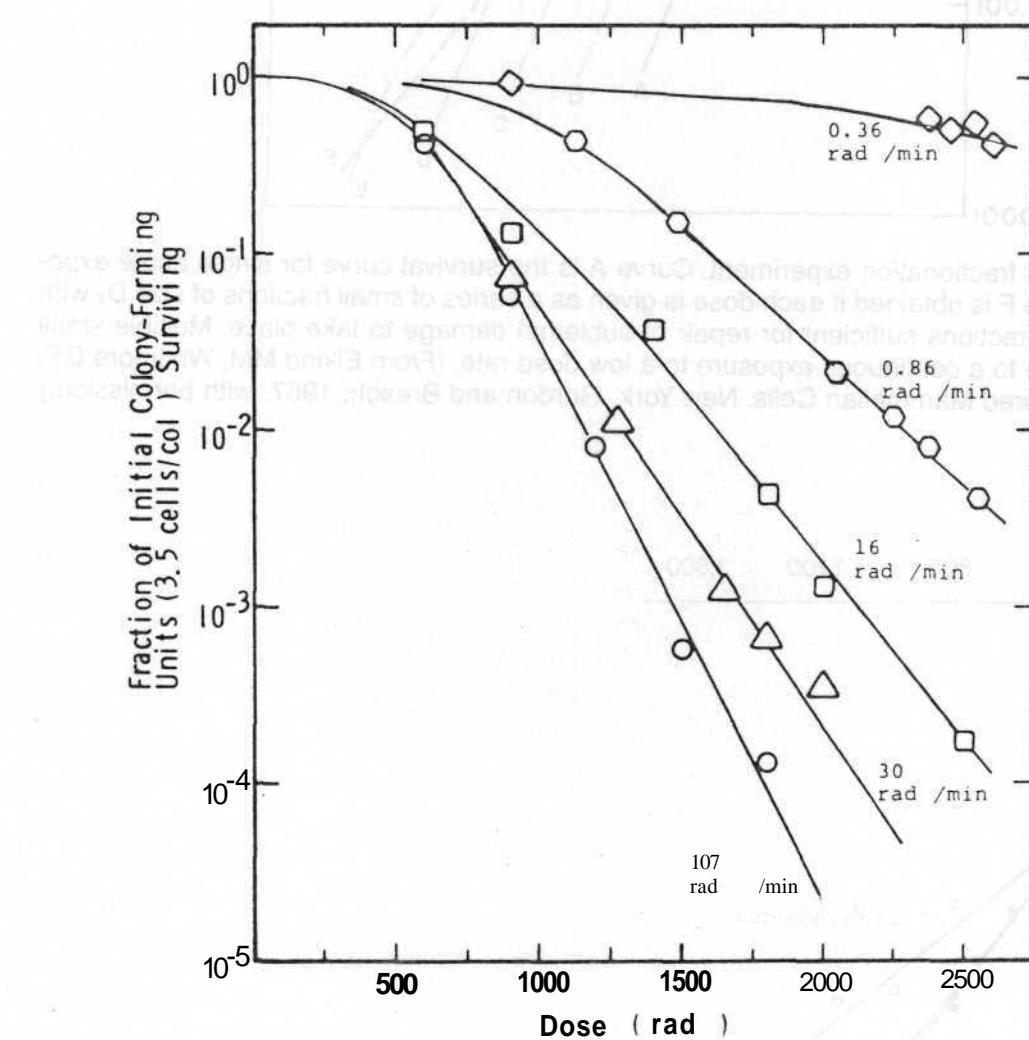


**Figure 5.9.** Survival curves for HeLa cells exposed to  $\gamma$ -rays at high and low dose rates.

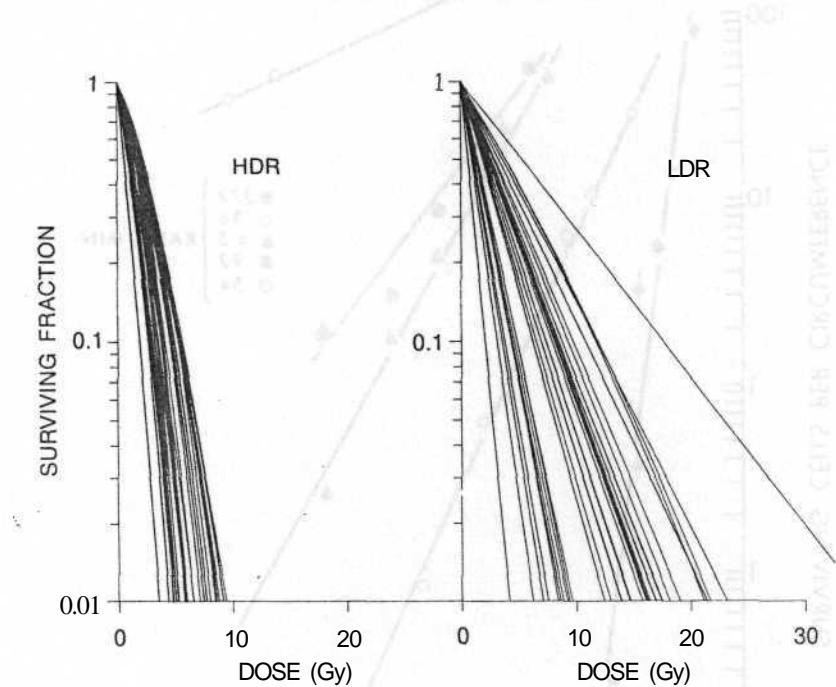
the dose rate is reduced, the survival curve becomes shallower and the shoulder tends to disappear (*i.e.*, the survival curve becomes an exponential function of dose). The dose-rate effect caused by repair of sublethal damage is most dramatic between 0.01 and 1 Gy/min (1 and 100 rad/min). Above and below this dose-rate range, the survival curve changes little, if at all, with dose rate.

The magnitude of the dose-rate effect from the repair of sublethal damage varies enormously among different types of cells. HeLa cells are characterized by a survival curve for

acute exposures that has a small initial shoulder, which goes hand in hand with a modest dose-rate effect. This is to be expected, because both are expressions of the cell's capacity to accumulate- and repair sublethal radiation damage. By contrast, Chinese hamster cells have a broad shoulder to their acute x-ray survival curve and show a correspondingly large dose-rate effect. This is evident in Figure 5.10; there is a clear-cut difference in biologic effect, at least at high doses, between dose rates of 1.07, 0.30, and 0.16 Gy/min (107, 30, and 16 rad/min). The differences be-



**Figure 5.10.** Dose-response curves for Chinese hamster cells (CHL-F line) grown *in vitro* and exposed to cobalt-60  $\gamma$ -rays at various dose rates. At high doses a substantial dose-rate effect is evident even among 1.07, 0.3, and 0.16 Gy/min (107, 30, and 16 rad/min). The decrease in cell killing becomes even more dramatic as the dose rate is reduced further. (From Bedford JS, Mitchell JB: Dose rate effects in synchronous mammalian cells in culture. Radiat Res 54:316-327, 1973, with permission.)



**Figure 5.11.** Dose-survival curves at high dose rates (HDR) and low dose rates (LDR) for a large number of cells of human origin cultured *in vitro*. Note that the survival curves fan out at low dose rates because in addition to a range of inherent radiosensitivities (evident at HDR) there is also a range of repair times of sublethal damage.

tween HeLa and hamster cells in the size of the shoulder to the acute survival curve and the magnitude of the dose-rate effect reflect differences in the importance of apoptosis. In the case of HeLa cells, apoptosis is an important form of cell death following radiation, whereas for hamster cells, apoptotic death is rarely seen.

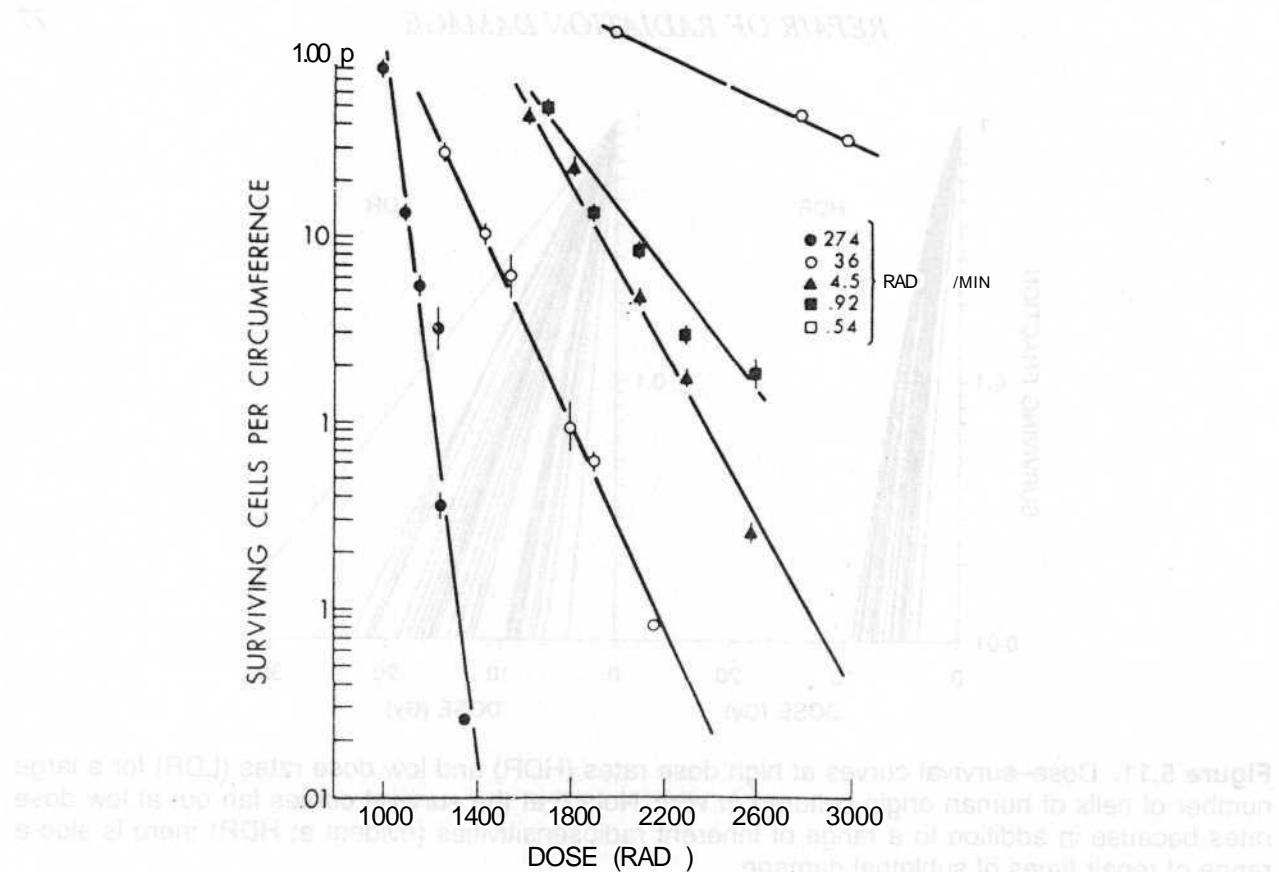
Figure 5.11 shows survival curves for 40 different cell lines of human origin, cultured *in vitro* and irradiated at a high dose rate and a low dose rate. At a low dose rate the survival curves "fan out" and show a greater variation in slope because, in addition to the variation of inherent radiosensitivity evident at a high dose rate, there is a range of repair times of sublethal damage. Some cell lines repair sublethal damage rapidly, some more slowly, and this is reflected in the survival curves at a low dose rate.

Survival curves for crypt cells in the mouse jejunum irradiated with  $\gamma$ -rays at various dose rates are shown in Figure 5.12.

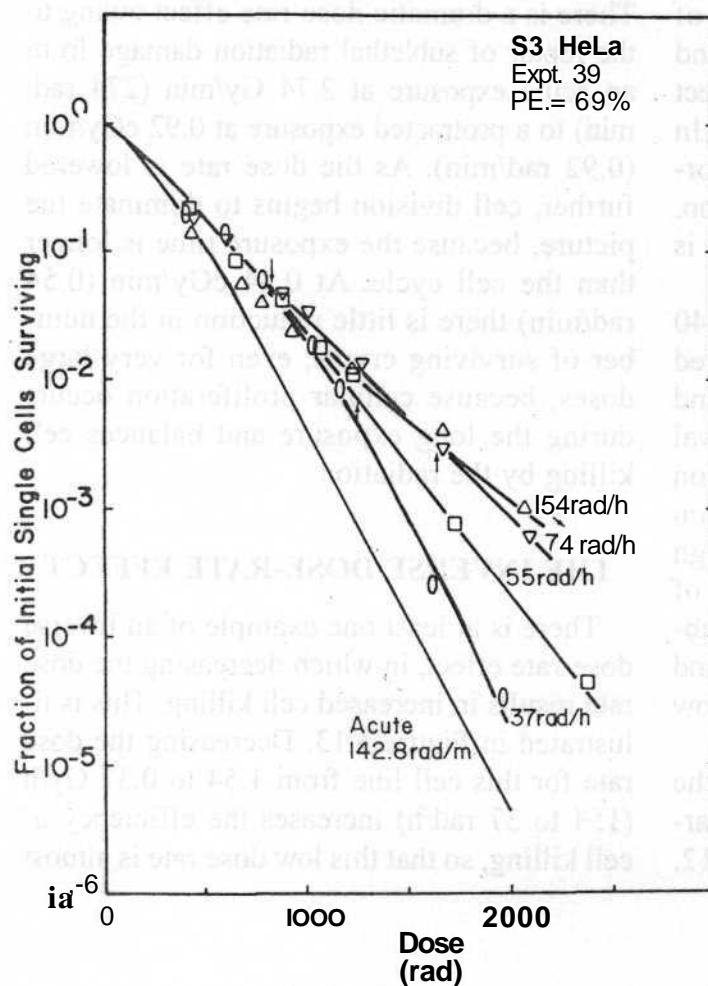
There is a dramatic dose-rate effect owing to the repair of sublethal radiation damage from an acute exposure at 2.74 Gy/min (274 rad/min) to a protracted exposure at 0.92 cGy/min (0.92 rad/min). As the dose rate is lowered further, cell division begins to dominate the picture, because the exposure time is longer than the cell cycle. At 0.54 cGy/min (0.54 rad/min) there is little reduction in the number of surviving crypts, even for very large doses, because cellular proliferation occurs during the long exposure and balances cell killing by the radiation.

### THE INVERSE DOSE-RATE EFFECT

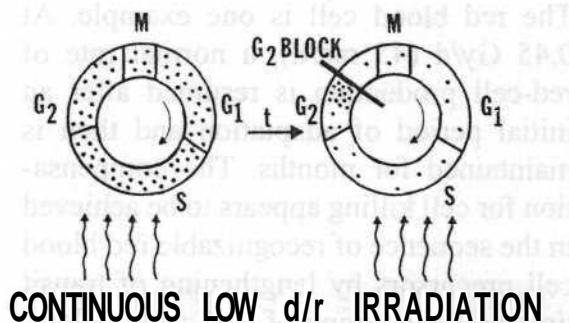
There is at least one example of an inverse dose-rate effect, in which decreasing the dose rate results in increased cell killing. This is illustrated in Figure 5.13. Decreasing the dose rate for this cell line from 1.54 to 0.37 Gy/h (154 to 37 rad/h) increases the efficiency of cell killing, so that this low dose rate is almost



**Figure 5.12.** Response of mouse jejunal crypt cells irradiated with  $\gamma$ -rays from cesium-137 over a wide range of dose rates. The mice were given total-body irradiation, and the proportion of surviving crypt cells was determined by the appearance of regenerating microcolonies in the crypts 3 days later. Note the large dose-rate effect. (From Fu K, Phillips TL: Radiology 114:709-716, 1975, with permission.)



**Figure 5.13.** The inverse dose-rate effect. A range of dose rates can be found for HeLa cells such that lowering the dose rate leads to more cell killing. At 1.54 Gy/h (154 rad/h), cells are "frozen" in the various phases of the cycle and do not progress. As the dose rate is dropped to 0.37 Gy/h (37 rad/h), cells progress to a block in G2, a radiosensitive phase of the cycle. (From Mitchell JB, Bedford JS, Bailey SM: Dose-rate effects on the cell cycle and survival of S3 HeLa and V79 cells. Radiat Res 79: 520-536, 1979, with permission.)

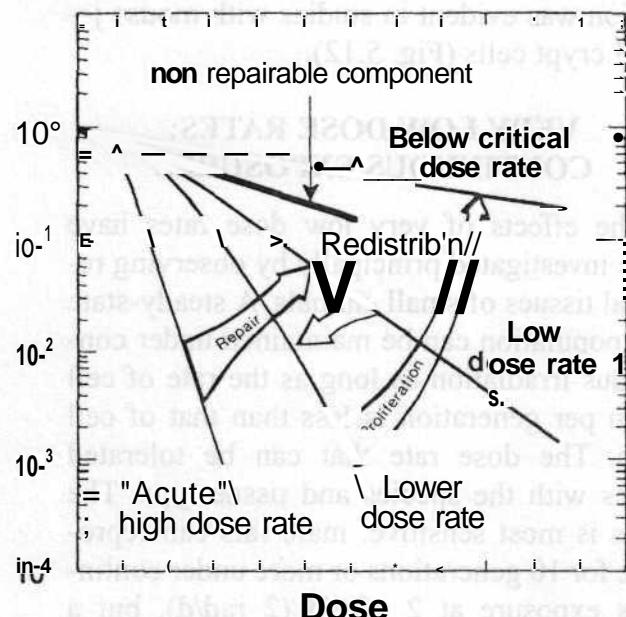


**Figure 5.14.** The inverse dose-rate effect. A range of dose rates can be found, at least for HeLa cells, that allows cells to progress through the cycle to a block in late G<sub>2</sub>. Under continuous low dose rate irradiation, an asynchronous population becomes a population of radiosensitive G<sub>2</sub> cells. (From Hall EJ: The biological basis of endocurietherapy: The Henschke Memorial Lecture 1984. Endocurie Hypertherm Oncol 1:141-151, 1985, with permission.)

as effective as an acute exposure. The explanation of this is illustrated in Figure 5.14. At about 0.3 Gy/h (30 rad/h), cells tend to progress through the cycle and become arrested in G<sub>2</sub>, a radiosensitive phase of the cycle. At higher dose rates they are "frozen" in the phase of the cycle they are in at the start of the irradiation; at lower dose rates they continue to cycle during irradiation.

#### THE DOSE-RATE EFFECT SUMMARIZED

Figure 5.15 summarizes the entire dose-rate effect. For acute exposures at high dose rates the survival curve has a significant initial shoulder. As the dose rate is lowered and the treatment time protracted, more and more sublethal damage can be repaired during the exposure. Consequently, the survival curve becomes progressively shallower (D<sub>0</sub> increases), and the shoulder tends to disappear. A point is reached at which all sublethal damage is repaired, resulting in a limiting slope. In at least some cell lines a further lowering of the dose rate allows cells to progress through the cycle and accumulate in G<sub>2</sub>. This is a ra-



**Figure 5.15.** The dose-rate effect resulting from repair of sublethal damage, redistribution in the cycle, and cell proliferation. The dose-response curve for acute exposures is characterized by a broad initial shoulder. As the dose rate is reduced, the survival curve becomes progressively shallower as more and more sublethal damage is repaired but cells are "frozen" in their positions in the cycle and do not progress. As the dose rate is lowered further and for a limited range of dose rates, the survival curve steepens again because cells can progress through the cycle to pile up at a block in G<sub>2</sub>, a radiosensitive phase, but still cannot divide. A further lowering of dose rate allows cells to escape the G<sub>2</sub> block and divide; cell proliferation then may occur during the protracted exposure, and survival curves become shallower as cell birth from mitosis offsets cell killing from the irradiation. (Based on the ideas of Dr. Joel Bedford.)

diosensitive phase, and so the survival curve becomes steeper again. This is the inverse dose-rate effect. A further reduction in dose rate allows cells to pass through the G<sub>2</sub> block and divide. Proliferation then may occur during the radiation exposure if the dose rate is low enough and the exposure time is long compared with the length of the mitotic cycle. This may lead to a further reduction in biologic effect as the dose rate is progressively lowered, because cell birth tends to balance cell death. A dose-rate effect from cell prolif-

eration was evident in studies with mouse jejunal crypt cells (Fig. 5.12).

### VERY LOW DOSE RATES: CONTINUOUS EXPOSURES

The effects of very low dose rates have been investigated principally by observing renewal tissues of small animals. A steady-state cell population can be maintained under continuous irradiation as long as the rate of cell death per generation is less than that of cell birth. The dose rate that can be tolerated varies with the species and tissue type. The testis is most sensitive: male rats can reproduce for 10 generations or more under continuous exposure at 2 cGy/d (2 rad/d), but a slight increase in dose rate above this level results in a depletion of the testis-cell population. At the other extreme, the small intestine in the rat has been shown to maintain cell division and a steady-state population at dose rates as high as 4 Gy/d (400 rad/d). The blood-forming tissues are intermediate between these two extremes; red blood cell production in the rat can be maintained at close to normal levels for months during exposure to 0.5 Gy/d (50 rad/d).

Lamerton and Courtenay drew attention to three principal factors that determine the response of renewal tissues to continuous irradiation:

1. The cellular sensitivity of the stem cells involved. Cells that exhibit an acute exposure survival curve with a broad shoulder would be expected to be less susceptible to low dose rate irradiation, because the shoulder is reconstructed continuously during a protracted exposure.
2. The duration of the cell cycle. The accumulated dose over the cell cycle is a more appropriate indicator of cell lethality than the dose rate. Thus a given dose rate of continuous irradiation is more damaging to cells with long cell cycles than to cells with short cycles, because a larger dose is absorbed per cell cycle.
3. The ability of some tissues to adapt to the new trauma of continuous irradiation.

The red blood cell is one example. At 0.45 Gy/d (45 rad/d), a normal rate of red-cell production is resumed after an initial period of adaptation and then is maintained for months. The compensation for cell killing appears to be achieved in the sequence of recognizable fed blood cell precursors by lengthening of transit time and shortening of cell cycle, allowing extra divisions to be inserted. The small intestine of the rat adapts rapidly to a continuous exposure of 3.5 Gy/d (350 rad/d). The cell cycle is lengthened within 6 hours of the start of irradiation, but this trend is reversed after 24 hours, and the cell cycle rapidly becomes shorter than normal.

### BRACHYTHERAPY OR ENDOCURIETHERAPY

Implanting radioactive sources directly into a tumor was a strategy first suggested by Alexander Graham Bell soon after the turn of the century. Over the years, various groups in different countries coined various names for this type of therapy, using the prefix *brachy-* from the Greek for "short range," or *endo-* from the Greek for "within." There are two distinct forms of **brachytherapy**: (1) *intracavitary* irradiation using radioactive sources that are placed in body cavities in close proximity to the tumor and (2) *interstitial* brachytherapy using radioactive seeds implanted directly into the tumor volume.

Both interstitial and intracavitary techniques were developed to an advanced stage at an early date because the technology was readily available. Radium in sufficient quantities was extracted and purified in the early 1900s, whereas radioactive sources of sufficient activity for teletherapy sources of adequate dose rate only came as a spin-off of World War II nuclear technology.

#### Intracavitary Therapy

Intracavitary radiotherapy at a low dose rate is always temporary and usually takes 1 to 4

days (dose rate about 50 cGy/h or 50 rad/h). It can be used for a number of anatomic sites, but by far the most common is the uterine cervix. There has been a continual evolution in the radionuclide used; in the early days radium was used, but this went out of favor because of the safety aspects of using an encapsulated source that can leak radioactivity. As an interim measure cesium-137 was introduced, but today most centers use iridium-192; its shorter half-life and lower energy make for ease of radiation protection, especially in conjunction with a remote afterloader.

To an increasing extent, low dose rate intracavitary therapy is being replaced by high dose rate intracavitary therapy, delivered in 3 to 12 dose fractions. Replacing continuous low dose rate therapy with a few large-dose fractions gives up much of the radiobiologic advantage and the sparing of late-responding normal tissues, as described in Chapter 22. It is only possible because the treatment of carcinoma of the cervix is a special case in which the dose-limiting normal tissues (*e.g.*, bladder, rectum) receive a lower dose than the prescribed dose to the tumor (or to point A). For high dose rate treatments lasting a few minutes, it is possible to use retractors that result in even lower doses to the critical normal tissues than are possible with an insertion that lasts 24 hours or more. These physical advantages offset the radiobiologic disadvantages, so that the general principle that a few large fractions at a high dose rate gives poorer results than at a low dose rate does not apply to this special case.

### Interstitial Therapy

Interstitial brachytherapy can be either temporary or permanent. Permanent implants in earlier times utilized radium, but the most widely used radionuclide at the present time is iridium-192. Implants at low dose rates are considered by many radiotherapists to be the treatment of choice for the 5% or so of human cancers that are accessible to such techniques.

The dose-rate range used in these treatments is in the region of the dose-rate spec-

trum in which the biologic effect varies rapidly with dose rate. The maximum dose that can be delivered without unacceptable damage to the surrounding normal tissue depends on the volume of tissue irradiated and on the dose rate, which is in turn a function of the number of radioactive sources used and their geometric distribution. To achieve a consistent biologic response, the total dose used should be varied according to the dose rate employed.

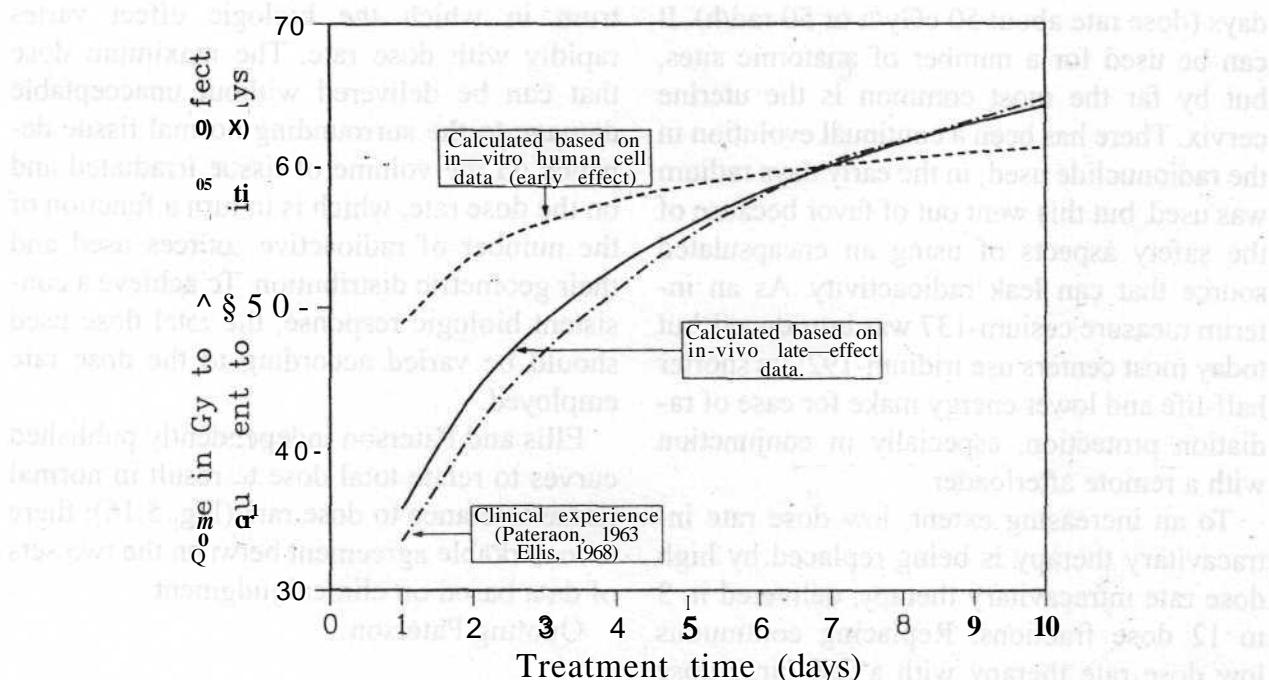
Ellis and Paterson independently published curves to relate total dose to result in normal tissue tolerance to dose rate (Fig. 5.16); there is remarkable agreement between the two sets of data based on clinical judgment.

Quoting Paterson:

The graph for radium implants is an attempt to set out the doses in five to ten days which are equivalent to any desired seven-day dose. In its original form it perhaps owed more to inspiration than to science but it has gradually been corrected to match actual experience.

Both Paterson and Ellis regarded a dose of 60 Gy (6,000 rad) in 7 days as the standard treatment, corresponding to a dose rate of 0.357 Gy/h (35.7 rad/h). If the sources are of higher activity and the treatment dose rate is higher, then a lower total dose should be used. For example, a dose rate of 0.64 Gy/h (64 rad/h) would produce an equivalent biologic effect with a total dose of only 46 Gy (4,600 rad) in a treatment time of 3 days.

Also shown in Figure 5.16 are isoeffect curves, matched to 60 Gy in 7 days, based on radiobiologic data for early- and late-responding tissues. The variation of total dose with dose rate is much larger for late- than for early-responding tissues because of the lower *o/c* characteristic of such tissues. It is interesting to note that the curve for late-responding tissues calculated from radiobiologic data agrees closely with the clinical estimates of Ellis and of Paterson, as it should, because their judgment was stated unequivocally to be based on late equalizing effects. In recent years, Mazeron and his colleagues in Paris have published two papers that show clearly



**Figure 5.16.** Dose equivalent to 60 Gy (6,000 rad.) in 7 days as proposed by Paterson (in 1963) and by Ellis (in 1968) based on clinical observation of normal tissue tolerance or calculated from radiobiologic principles. The  $\alpha/\beta$  ratios and the T1/2 for repair of sublethal damage were chosen for early or for late-responding tissues (see Chapter 22).

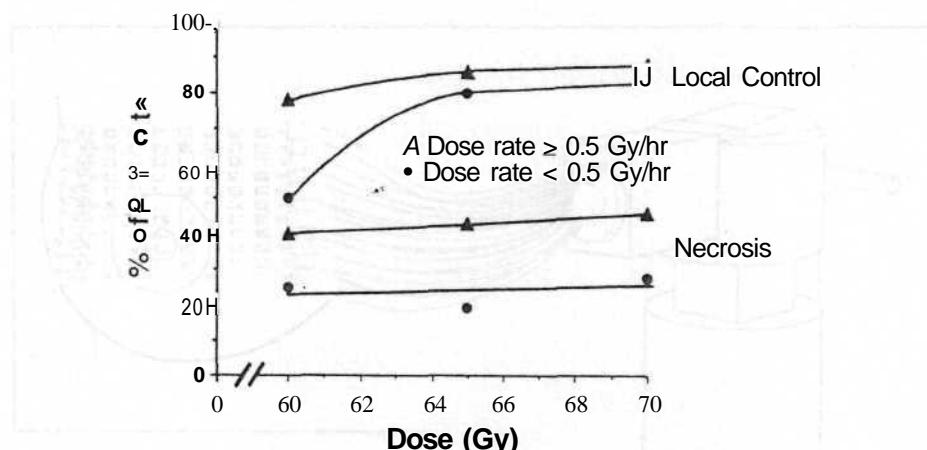
that a dose-rate effect is important in interstitial implants. They have, perhaps, the largest experience in the world of the use of iridium-192 wire implants.

Their first report describes the analysis of local tumor control and the incidence of necrosis in a large cohort of patients with T1-2 squamous cell carcinoma of the mobile tongue and the floor of the mouth who were treated with interstitial iridium-192. The data are shown in Figure 5.17. Patients were grouped according to dose rate, either more or less than 0.5 Gy/h (50 rad/h). It is evident that there is a substantially higher incidence of necrosis in patients treated at the higher dose rates. By contrast, dose rate makes little or no difference to local control provided the total dose is high enough, 65 to 70 Gy (6,500-7,000 rad), but there is a clear separation at lower doses (60 Gy or 6,000 rad), with the lower dose rate being less effective. These results are in good accord with the radiobiologic predictions.

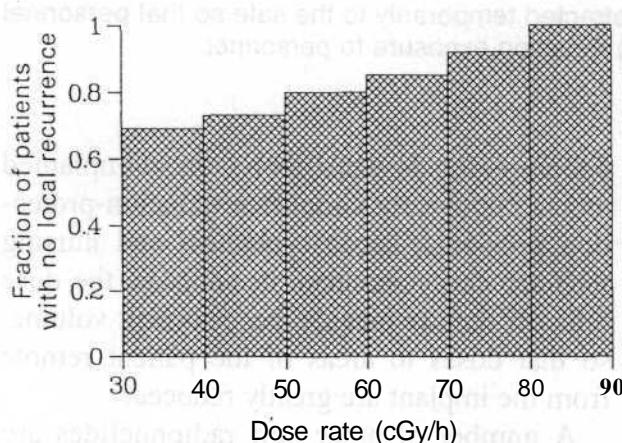
The second report analyzes data from a large group of patients with carcinoma of the

breast who received iridium-192 implants as a boost to external-beam radiotherapy. These results allow an assessment of the effect of dose rate on tumor control but provide no information on the effect of dose rate on late effects, because there was only one case that involved necrosis. The interstitial implant was only part of the radiotherapy, and a fixed standard dose was used, so only limited conclusions can be drawn from these data. The results (Fig. 5.18), however, show a correlation between the proportion of recurrent tumors and the dose rate. For a given total dose, there were markedly fewer recurrences if the radiation was delivered at a higher dose rate rather than a lower dose rate.

The relatively short half-life of iridium 192 (70 days) means that a range of dose rates is inevitable, because the activity of the sources decays during the months that they are in use. It is important, therefore, to correct the total dose for the dose rate, because of the experience of Mazeran and his colleagues described previously. Iridium-192 has two advantages: (1) The source size can be small, and (2) its



**Figure 5.17.** Local tumor control and necrosis rate at 5 years as a function of dose in patients treated for T1-2 squamous-cell carcinomas of the mobile tongue and the floor of the mouth with interstitial iridium-192 implants. The patients were grouped according to whether the implant was characterized by a high dose rate (above 0.5 Gy/h or 50 rad/h) or low dose rate (below 0.5 Gy/h or 50 rad/h). The necrosis rate is higher for the higher dose rate at all dose levels. Local tumor control did not depend on dose rate provided the total dose was sufficiently large. (Data from Mazeron JJ, Simon JM, Le Pechoux C, et al: Effect of dose rate on local control and complications in definitive irradiation of T1-2 squamous cell carcinomas of mobile tongue and floor of mouth with interstitial iridium-192. *Radiother Oncol* 21:39-47, 1991.)

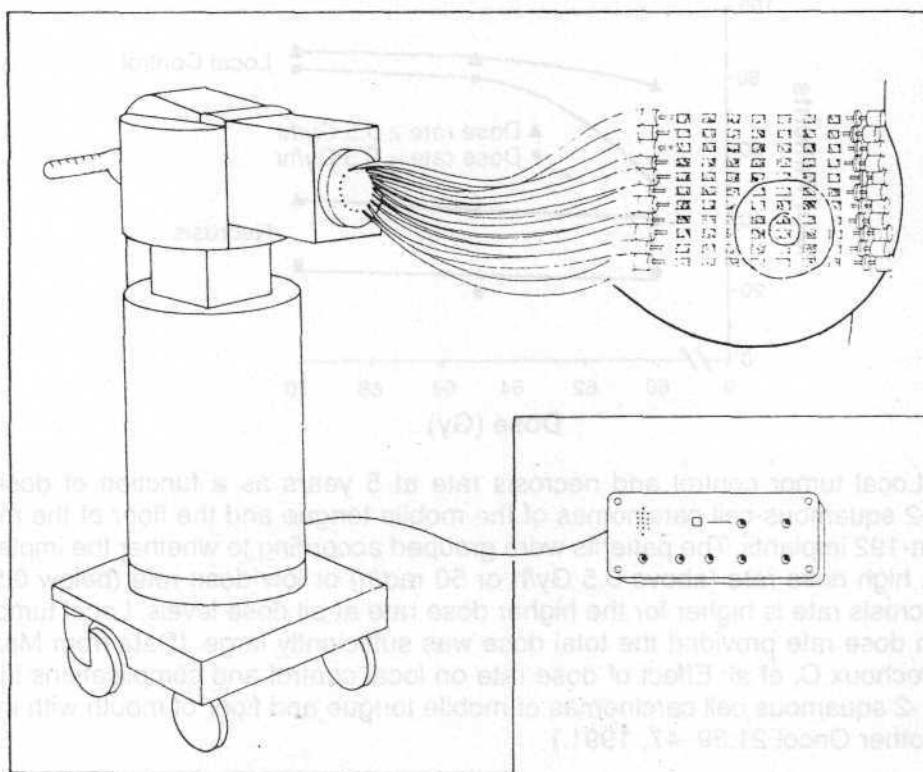


**Figure 5.18.** Percentage of patients who showed no local recurrence as a function of dose rate in treatment for breast carcinoma by a combination of external-beam irradiation plus iridium-192 interstitial implant. The implant was used to deliver a dose of 37 Gy (3,700 rad); the dose rate varied by a factor of 3, owing to different linear activities of the iridium-192 wire and different volumes implanted. (Data from Mazeron JJ, Simon JM, Crook J, et al: Influence of dose rate on local control of breast carcinoma treated by external beam irradiation plus iridium-192 implant. *Int J Radiat Oncol Biol Phys* 21: 1173-1177, 1991.)

lower photon energy makes protection easier than with radium or cesium-137. Sources of this radionuclide are ideal for use with a computer-controlled afterloader (Fig. 5.19). Catheters can be implanted into the patient while inactive, and then the sources transferred from the safe by remote control after the patient has returned to his own room. The sources can be returned to the safe if the patient needs nursing care.

### Permanent Interstitial Implants

Encapsulated sources with relatively short half-lives can be left in place permanently. There are two advantages for the patient: (1) An operation to remove the implant is not needed, and (2) the patient can go home with the implant in place. On the other hand, this does involve additional expense, because the sources are not reused. The initial dose rate is high and falls off as the implanted sources decay. Iodine-125 has been used most widely to date for permanent implants. The total prescribed dose is usually about 160 Gy (16,000 rad) at the periphery of the implanted volume, with 80 Gy (8,000 rad) delivered in the first



**Figure 5.19.** Diagram illustrating the use of a computer-controlled remote afterloader to minimize radiation exposure of personnel during brachytherapy. Catheters are implanted into the tumor, and radiographs are made to check the validity of the implant using "dummy" nonradioactive sources. The catheters then are connected to a shielded safe containing the radioactive (iridium-192) sources, which are transferred by remote control to the implant in the patient. The control panel is located outside a lightly shielded room. The sources can be retracted temporarily to the safe so that personnel can care for the patient, thus effectively eliminating radiation exposure to personnel.

half-life of 60 days. The soft emission from iodine has a relative biologic effectiveness of about 1.5; this corresponds to  $80 \times 1.5$  or 120 Gy (12,000 rad) of high-energy  $\alpha$ -rays. This is a big dose, even at a low dose rate, and corresponds to a good level of cell kill. It is, however, spread over 60 days, and consequently the success of the implant in sterilizing the tumor depends critically on the cell cycle of the clonogenic cells. In a rapidly growing tumor, cell birth by mitosis compensates for cell killing by the radiation during the prolonged exposure time. This is much less of a problem with slowly growing tumors, such as carcinoma of the prostate, and it is in such sites that permanent implants with iodine-125 have found a place.

A major advantage of a radionuclide such as iodine-125 is the low energy of the photons emitted (about 30 keV). This makes little dif-

ference to the dose distribution in an implanted tumor but greatly simplifies radiation-protection problems, because medical and nursing staff are easily shielded. In addition, the dose falls off rapidly outside the treatment volume, so that doses to areas of the patient remote from the implant are greatly reduced.

A number of other new radionuclides are under consideration as sources for brachytherapy that share with iodine-125 the properties of a relatively short half-life and a low-energy photon emission to reduce problems of radiation protection. By contrast, americium-241 emits a low-energy photon but has a long half-life of hundreds of years. Table 5.1 summarizes some of the physical characteristics of the newly developed sources and contrasts them with the characteristics of radionuclides more commonly used for brachytherapy.

TABLE 5.1. Characteristics of Radionuclides for Intracavitary or Interstitial Brachytherapy

Radionuclide	Photon Energy, KeV		Half-life	HVL, mm lead
	Average	Range		
<b>Conventional</b>				
Cesium-137	662	—	30 y	5.5
Iridium-192	380	136-1060	74.2 d	2.5
<b>New</b>				
Iodine-125	28	3-35	60.2 d	0.025
Gold-198	412	—	2.7 d	2.5
Americium-241	60	—	432 y	0.125
Palladium-103	21	20-23	17 d	0.008
Samarium-145	41	38-61	340 d	0.06
Ytterbium-169	100	10-308	32 d	0.1

Data computed by Dr. Ravinder Nath, Yale University.

## RADIOLABELED IMMUNOGLOBULIN THERAPY FOR HUMAN CANCER

Radiolabeled immunoglobulin therapy is radiotherapy for cancer using an antibody to deliver a radioactive isotope to the tumor. Much of the pioneering work in this field has been done by Stanley Order and his colleagues. They have focused on antiferritin labeled with radioactive iodine or yttrium.

Ferritin is an iron-storage protein that is synthesized and secreted by a broad range of malignancies, including hepatoma, lung cancer, neuroblastoma, acute myelogenous leukemia, cancer of the breast and pancreas, and Hodgkin's disease. It is not known why ferritin is produced preferentially in tumors. It has been suggested that messenger RNA for ferritin may resemble that for many viruses. This suggestion is highly speculative but consistent with the observation that ferritin is present in tumors that are suspected of having a viral cause. This connection is strongly suspected for hepatomas, which have been associated with hepatitis B virus, and probably exists for Hodgkin's disease, too.

Although ferritin is also present in normal tissues, selective tumor targeting has been demonstrated in animal models and in clinical scanning, historically performed first for Hodgkin's disease. This differential is the basis of the potential therapeutic gain and, therefore, the clinical usefulness of radiolabeled immunoglobulin therapy.

In the early years of radiolabeled immunoglobulin therapy, radiolabeled polyclonal antibodies were used. These were replaced with murine monoclonal antibodies carrying iodine-131, which could be used for both diagnosis and therapy. More recently, chimeric mouse-human antibodies, which are human antibodies derived by tissue culture or produced in genetically altered mice, and synthetically derived antibodies have become available. These developments have progressively reduced the possibility of inducing an immune response, lengthened the effective half-life, and hence increased the tumor dose.

### Radionuclides

Early studies utilized iodine-131, which is easily linked to antibodies. The disadvantage of using iodine-131 is that it requires large doses (about 30 mCi); as a consequence of this, patients must be hospitalized, self-care is needed, and pediatric patients are excluded. In addition, the dose and dose rate to the tumor are limited by the relatively weak ( $\beta$ -emission (0.3 MeV) and by the total-body dose resulting from the  $\gamma$ -emission, which causes systemic hematopoietic toxicity.

In more recent developments, iodine-131 has been replaced by yttrium-90, which is characterized by a pure ( $\beta$ -emission of relatively high energy (0.9 MeV). This allows a higher tumor dose and dose rate and enables

the applications to be administered on an out-patient basis. More recently, rhenium-188, rhenium-186, and phosphorus-32 all have been used. New chemical linkages, including a variety of chelates, have been used, all seeking to bind the isotope firmly to the antibody.

### Tumor Target Visualization

When iodine-131 was used, the  $\gamma$ -ray emission allowed tumor localization as well as provided the bulk of the therapeutic dose. When pure  $\beta$ -emitters such as yttrium-90 were first introduced, it was necessary to add a  $\gamma$ -emitter such as indium-111 to allow visualization. Today, it is no longer acceptable to scan with a conventional  $\gamma$ -camera, because single photon emission computed tomography provides a clearer picture. The bremsstrahlung from  $\beta$ -emitters can be scanned by this means, so that radionuclides such as yttrium-90 can be used without the need to add a  $\gamma$ -emitter for visualization.

### Targeting

The ability to target tumors with antiferritin mirrors the vascularity of the tumor nodules. In general, tumors with a high degree of vascularity are better targeted with antiferritin than less vascularized tumors. The presence of ferritin *per se* is not enough to ensure targeting. The need for neovasculature means that uptake tends to be greater in smaller tumors. Uptake also can be affected by radiation or hyperthermia. A dose of external radiation can act as an initiator. This first was observed empirically but now is used routinely to enhance the targeting of the radiolabeled antiferritin. This is probably a consequence of damage to tumor vasculature, which allows antiferritin to leak out of vessels and into tumor cells. The targeting ratio of a tumor to the average for normal tissue is about 2.9 for antiferritin labeled with iodine-131; the corresponding ratios are 1.2 for bone and gastrointestinal tract and 0.8 for lung.

### Clinical Results

The most promising results have been in the treatment of unresectable primary hepatoma, for which 48% partial remission and 7% complete remission rates have been reported by the Johns Hopkins group for patients receiving iodine-131-labeled antiferritin in combination with low doses of doxorubicin (Adriamycin) and 5-fluorouracil. Some success also has been reported by other groups using similar techniques in the treatment of metastatic neuroblastoma, relapsed grade IV gliomas after radiotherapy and chemotherapy, metastatic ovarian cancer resistant to prior radiotherapy, and malignant pleura! and pericardial effusions of diverse causes.

Iodine-131-labeled antiferritin led to partial remissions in patients with Hodgkin's disease, but yttrium-90 antiferritin produced complete remissions, indicating the increased effectiveness of the larger doses possible with radionuclides emitting pure  $\beta$ -rays. Radiolabeled immunoglobulin therapy has been used with varying success for a wide range of other malignancies, including hepatomas, ovarian cancer, gliomas, and leukemia. Although a variety of radiolabeled antibodies has been shown to achieve remissions in lymphoma, the question of the effect of the total-body exposure versus tumor targeting is still open.

### Dosimetry

For iodine-131-labeled antiferritin treatment of primary hepatoma, 30 mCi is administered on day 0, followed by 20 mCi on day 5. Escalation of dose beyond these levels is not helpful, because the deposition of labeled antiferritin becomes saturated. This translates into a peak dose rate of 45 to 50 mGy/h (4.5 to 5 rad/h) on days 1 and 5 and a total accumulated dose of 10 to 12 Gy (1,000-4,200 rad) by about 15 days. The corresponding dose rate to normal liver is 10 mGy/h (1 rad/h), and the total-body dose is 2 to 3 mGy/h (0.2 to 0.3 rad/h), which results in limiting hematologic toxicity. It is remarkable

that such a small dose at such a low dose rate can produce remissions in patients with tumors of 1 kg or more. This response is difficult to explain on the basis of conventional radiobiologic data, but the clinical results are exciting.

For yttrium-90-labeled antiferritin treatment, a single application of 20 mCi results in a peak dose of about 0.16 Gy/h (16 rad/h), which decays with a tumor-effective half-life of 2 days and results in a total dose of about 20 to 35 Gy (2,000-3,500 rad).

### SUMMARY OF PERTINENT CONCLUSIONS

#### Potentially Lethal Damage

- The component of radiation damage that can be modified by manipulation of the postirradiation conditions is known as *potentially lethal damage*.
- Potentially lethal damage repair can occur if cells are prevented from dividing for 6 hours or more after irradiation; this is manifest as an increase in survival. This repair can be demonstrated *in vitro* by keeping cells in saline or plateau phase for 6 hours after irradiation and *in vivo* by delayed removal and assay of animal tumors or cells of normal tissues.
- Potentially lethal damage repair is significant for x-rays but does not occur after neutron irradiation.
- It has been suggested that resistant human tumors (*e.g.*, melanoma) owe their resistance to large amounts of potentially lethal damage repair. This is still controversial.

#### Sublethal Damage

- *Sublethal damage repair* is an operational term that describes the increase in survival if a dose of radiation is split into two fractions separated in time.
- The half-time of sublethal damage repair in mammalian cells is about 1 hour, but it may be longer in late-responding normal tissues *in vivo*.
- Sublethal damage repair occurs in tumors and normal tissues *in vivo* as well as in cells cultured *in vitro*.
- The repair of sublethal damage reflects the repair of DNA breaks before they can interact to form lethal chromosomal aberrations.
- Sublethal damage repair is significant for x-rays but almost nonexistent for neutrons.

#### Dose-rate Effect

- If the radiation dose rate is reduced from about 1 Gy/min to 0.3 Gy/h (100 rad/min to 30 rad/h), there is a reduction in the cell killing from a given dose, because sublethal damage repair occurs during the protracted exposure.
- As the dose rate is reduced, the slope of the survival curve becomes shallower ( $D_o$  increases), and the shoulder tends to disappear.
- In some cell lines an inverse dose-rate effect is evident (*i.e.*, reducing the dose rate increases the proportion of cells killed) owing to the accumulation of cells in G2, which is a sensitive phase of the cycle.

#### Brachytherapy

- Implanting sources into or close to a tumor is known as brachytherapy (from the Greek *brachy-*, meaning "short") or endocurietherapy (from *endo-*, meaning "within").

- Intracavitary radiotherapy involves placing radioactive sources into a body cavity close to a tumor. The most common example is the treatment of carcinoma of the uterine cervix.
- Interstitial therapy involves implanting radioactive sources directly into the tumor and adjacent normal tissue.
- Temporary implants, which formerly utilized radium needles, now are performed most often with iridium-192 wires or seeds.
- If the implant is used as a sole treatment, a commonly used dose is 50 to 70 Gy (5,000-7,000 rad) in 5 to 9 days. Total dose should be adjusted for dose rate. Clinical studies show that both tumor control and late effects vary with dose rate for a given total dose. Often the implant is used as a boost to external-beam therapy, and only half the treatment is given with the implant.
- Because of their small size and low photon energy, iridium-192 seeds are suitable for use with computer-controlled remote afterloaders.
- Permanent implants can be used with radionuclides (such as iodine-125 or palladium-103) that have relatively short half-lives.
- A number of novel radionuclides are being considered as sources for brachytherapy. Most emit low-energy photons, which simplifies the problems of radiation protection.

### Radiolabeled Immunoglobulin Therapy

- In the early days of radiolabeled immunoglobulin therapy, radiolabeled polyclonal antibodies were used. These were replaced with murine monoclonal antibodies. More recently, chimeric mouse-human antibodies, which are human antibodies derived by tissue culture or produced in genetically altered mice, have become available. Finally, synthetically derived antibodies have been produced.
- Iodine-131 largely has been replaced by pure  $\beta$ -ray emitters such as yttrium-90, resulting in an increased tumor dose and decreased total-body toxicity.
- Single photon emission computed tomography can be used to visualize the tumor, using the bremsstrahlung from the  $\beta$ -rays.
- Radiolabeled immunoglobulin therapy has produced promising results in unresectable primary hepatoma and in patients with Hodgkin's lymphoma. It has been used with varying success for a wide range of other malignancies. These results have been obtained with total tumor doses of 20 to 35 Gy (2,000-3,500 rad) delivered at very low dose rates.

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# 6

## The Oxygen Effect and Reoxygenation

THE NATURE OF THE OXYGEN EFFECT  
THE TIME AT WHICH OXYGEN ACTS AND  
THE MECHANISM OF THE OXYGEN  
EFFECT  
THE CONCENTRATION OF OXYGEN  
REQUIRED  
CHRONIC AND ACUTE HYPOXIA  
THE FIRST EXPERIMENTAL  
DEMONSTRATION OF HYPOXIC CELLS  
IN A TUMOR  
PROPORTION OF HYPOXIC CELLS IN  
VARIOUS ANIMAL TUMORS

EVIDENCE FOR HYPOXIA IN HUMAN  
TUMORS  
OXYGEN PROBE MEASUREMENTS AS A  
PREDICTIVE ASSAY  
REOXYGENATION  
TIME SEQUENCE OF REOXYGENATION  
MECHANISM OF REOXYGENATION  
THE IMPORTANCE OF REOXYGENATION  
IN RADIOTHERAPY  
HYPOXIA AND TUMOR PROGRESSION  
SUMMARY OF PERTINENT CONCLUSIONS

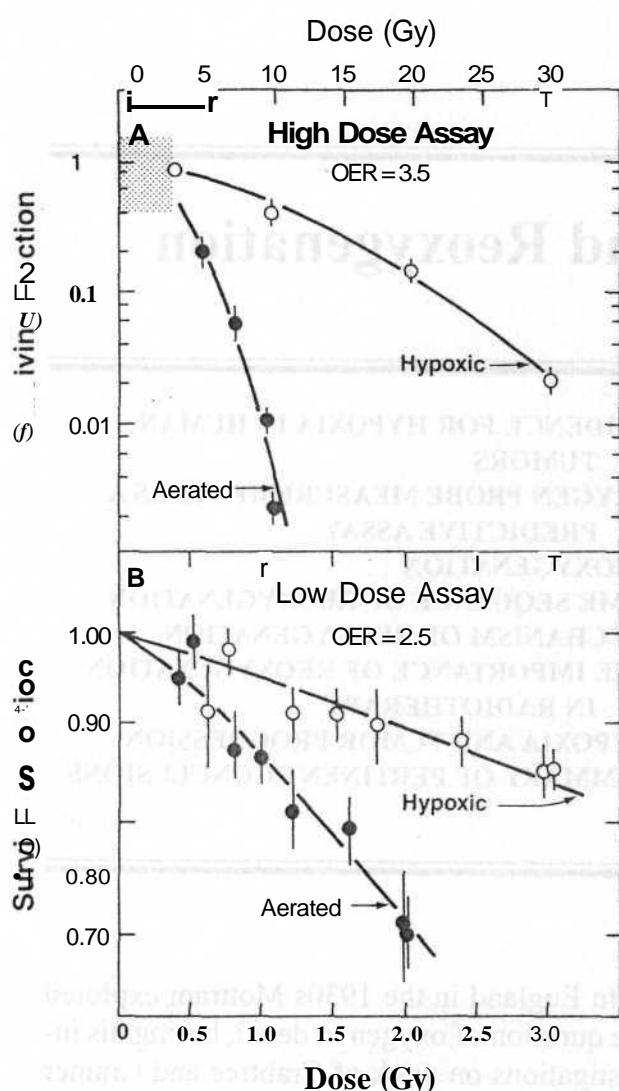
A host of chemical and pharmacologic agents that modify the biological effect of ionizing radiations have been discovered. None is simpler than oxygen, none produces such a dramatic effect, and, as it turns out, no other agent has such obvious practical implications.

The oxygen effect was observed as early as 1912 by Swartz in Germany, who noted that the skin reaction produced on his forearm by a radium applicator was reduced if the applicator was pressed hard onto the skin. He attributed this to the interruption in blood flow. By 1921 it had been noted by Holthusen that Ascaris eggs were relatively resistant to radiation in the absence of oxygen, a result wrongly attributed to the absence of cell division under these conditions. The correlation between radiosensitivity and the presence of oxygen was made by Petry in 1923 from a study of the effects of radiation on vegetable seeds. All of these results were published in the German literature but were apparently little known in the English-speaking world.

In England in the 1930s Mottram explored the question of oxygen in detail, basing his investigations on work of Crabtree and Cramer on the survival of tumor slices irradiated in the presence or absence of oxygen. He also discussed the importance of these findings to radiotherapy. Mottram began a series of experiments that culminated in a quantitative measurement of the oxygen effect by his colleagues Gray and Read, using as a biologic test system the growth inhibition of the primary root of the broad bean *Vicia faba*.

### THE NATURE OF THE OXYGEN EFFECT

Survival curves for mammalian cells exposed to x-rays in the presence and absence of oxygen are illustrated in Figure 6.1. The ratio of hypoxic to aerated doses needed to achieve the same biological effect is called the **oxygen enhancement ratio** (OER). For sparsely ionizing radiations, such as x- and y-rays, the



**Figure 6.1.** Cells are much more sensitive to x-rays in the presence of molecular oxygen than in its absence [*i.e.*, under hypoxia]. The ratio of doses under hypoxia to aerated conditions necessary to produce the same level of cell killing is called the oxygen enhancement ratio (OER). It has a value close to 3 at high doses but may have a lower value of about 2 at x-ray doses below about 2 Gy (200 rad). (Adapted from Palcic B, Skarsgard LD: Reduced oxygen enhancement ratio at low doses of ionizing radiation. Radiat Res 100:328-339, 1984, with permission.)

OER at high doses has a value of between 2.5 and 3. The OER has been determined for a wide variety of chemical and biologic systems with different endpoints, and its value for x-rays always tends to fall in this range. There is some evidence that for rapidly growing cells cultured *in vitro* the OER has a smaller value of about 2 at lower doses, of the order of the

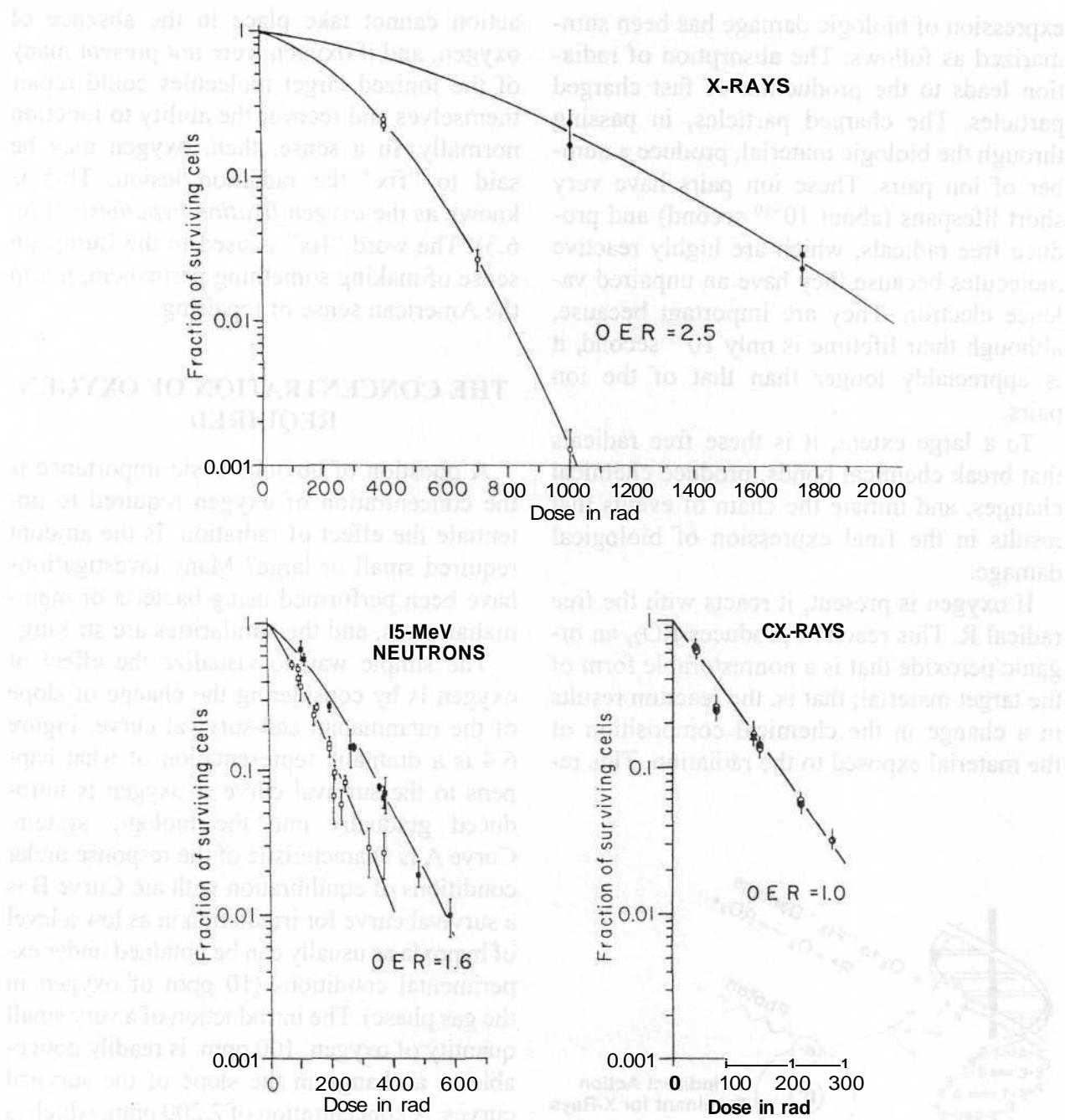
daily dose per fraction in radiotherapy. This is believed to result from the variation of OER with the phase of the cell cycle: Cells in G<sub>1</sub> have a lower OER than those in S, and because G<sub>1</sub> cells are more radiosensitive they dominate the low-dose region of the survival curve. For this reason the OER of an asynchronous population is slightly smaller at low doses than at high doses. This result has been demonstrated for a fast-growing cell cultured *in vitro*, for which precise survival measurements are possible, but would be difficult to show in a tissue. There is some evidence also in cells in culture that the survival curve has a complex shape for doses below 1 Gy (100 rad). What effect, if any, this has on the OER is not yet clear.

Figure 6.2 illustrates the oxygen effect for other types of ionizing radiations. For a densely ionizing radiation, such as low-energy  $\alpha$ -particles, the survival curve does not have an initial shoulder. In this case, survival estimates made in the presence or absence of oxygen fall along a common line; the OER is unity—in other words, there is no oxygen effect. For radiations of intermediate ionizing density, such as neutrons, the survival curves have a much reduced shoulder. In this case the oxygen effect is apparent, but it is much smaller than is the case for x-rays. In the example shown in Figure 6.2, the OER for neutrons is about 1.6.

In summary, the oxygen effect is large and important in the case of sparsely ionizing radiations, such as x-rays; is absent for densely ionizing radiations, such as  $\alpha$ -particles; and has an intermediate value for fast neutrons.

#### THE TIME AT WHICH OXYGEN ACTS AND THE MECHANISM OF THE OXYGEN EFFECT

For the oxygen effect to be observed, oxygen must be present during the radiation exposure or, to be precise, *during or within microseconds after* the radiation exposure. Sophisticated experiments have been performed in which oxygen, contained in a chamber at high pressure, was allowed to "ex-



**Figure 6.2.** The oxygen enhancement ratio (OER) for various types of radiation. The OER for a particles is unity. X-rays exhibit a larger OER of 2.5. Neutrons (15-MeV  $d^+ \rightarrow T$ ) are between these extremes, with an OER of 1.6. (Adapted from Barendsen GW, Koot CJ, van Kersen GR, Bewley DK, Field SW, Parnell CJ: Int J Radiat Biol 10:317, 1966; and Broerse JJ, Barendsen GW, van Kersen GR: Int J Radiat Biol 13:559, 1967, with permission.)

plode" onto a single layer of bacteria (and later mammalian cells) at various times before or after irradiation with a 2-jis electron pulse from a linear accelerator. It was found that oxygen need not be present during the irradiation to sensitize but could be added *af-terward*, provided the delay was not too long.

Some sensitization occurred **with** oxygen added as late as 5 ms after irradiation.

Experiments such as these shed some light on the mechanism of the oxygen effect. There is general agreement that oxygen acts at the level of the free radicals. The chain of events from the absorption of radiation to the final

expression of biologic damage has been summarized as follows: The absorption of radiation leads to the production of fast charged particles. The charged particles, in passing through the biologic material, produce a number of ion pairs. These ion pairs have very short lifespans (about  $10^{-10}$  second) and produce free radicals, which are highly reactive molecules because they have an unpaired valence electron. They are important because, although their lifetime is only  $10^{-5}$  second, it is appreciably longer than that of the ion pairs.

To a large extent, it is these free radicals that break chemical bonds, produce chemical changes, and initiate the chain of events that results in the final expression of biological damage.

If oxygen is present, it reacts with the free radical R. This reaction produces RO<sub>2</sub>, an organic peroxide that is a nonrestorable form of the target material; that is, the reaction results in a change in the chemical composition of the material exposed to the radiation. This re-

action cannot take place in the absence of oxygen, and if oxygen were not present many of the ionized target molecules could repair themselves and recover the ability to function normally. In a sense, then, oxygen may be said to "fix" the radiation lesion. This is known as the *oxygen fixation hypothesis* (Fig. 6.3). The word "fix" is used in the European sense of making something permanent, not in the American sense of repairing.

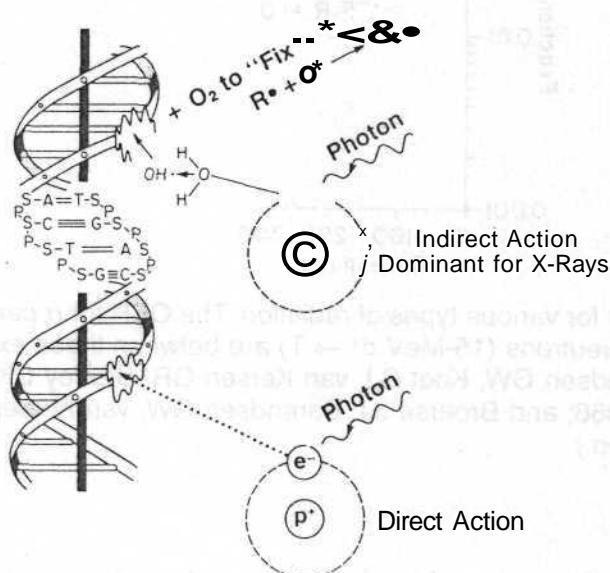
### THE CONCENTRATION OF OXYGEN REQUIRED

A question of obvious basic importance is the concentration of oxygen required to potentiate the effect of radiation. Is the amount required small or large? Many investigations have been performed using bacteria or mammalian cells, and the similarities are striking.

The simple way to visualize the effect of oxygen is by considering the change of slope of the mammalian cell-survival curve. Figure 6.4 is a dramatic representation of what happens to the survival curve if oxygen is introduced gradually into the biologic system. Curve A is characteristic of the response under conditions of equilibration with air. Curve B is a survival curve for irradiation in as low a level of hypoxia as usually can be obtained under experimental conditions (10 ppm of oxygen in the gas phase). The introduction of a very small quantity of oxygen, 100 ppm, is readily noticeable in a change in the slope of the survival curves. A concentration of 2,200 ppm, which is about 0.25% oxygen, moves the survival curve halfway toward the fully aerated condition.

Other experiments have shown that by the time a concentration of oxygen corresponding to 2% has been reached, the survival curve is virtually indistinguishable from that obtained under conditions of normal aeration. Furthermore, increasing the amount of oxygen present from that characteristic of air to 100% oxygen does not further affect the slope of the curve.

The more usual textbook representation of the variation of radiosensitivity with oxygen concentration is shown in Figure 6.5. The term used here to represent radiosensitivity is pro-



**Figure 6.3.** The oxygen fixation hypothesis. About two thirds of the biologic damage produced by x-rays is by indirect action, mediated by free radicals. The damage produced by free radicals in DNA can be repaired under hypoxia but may be "fixed" (made permanent and irreparable) if molecular oxygen is available.

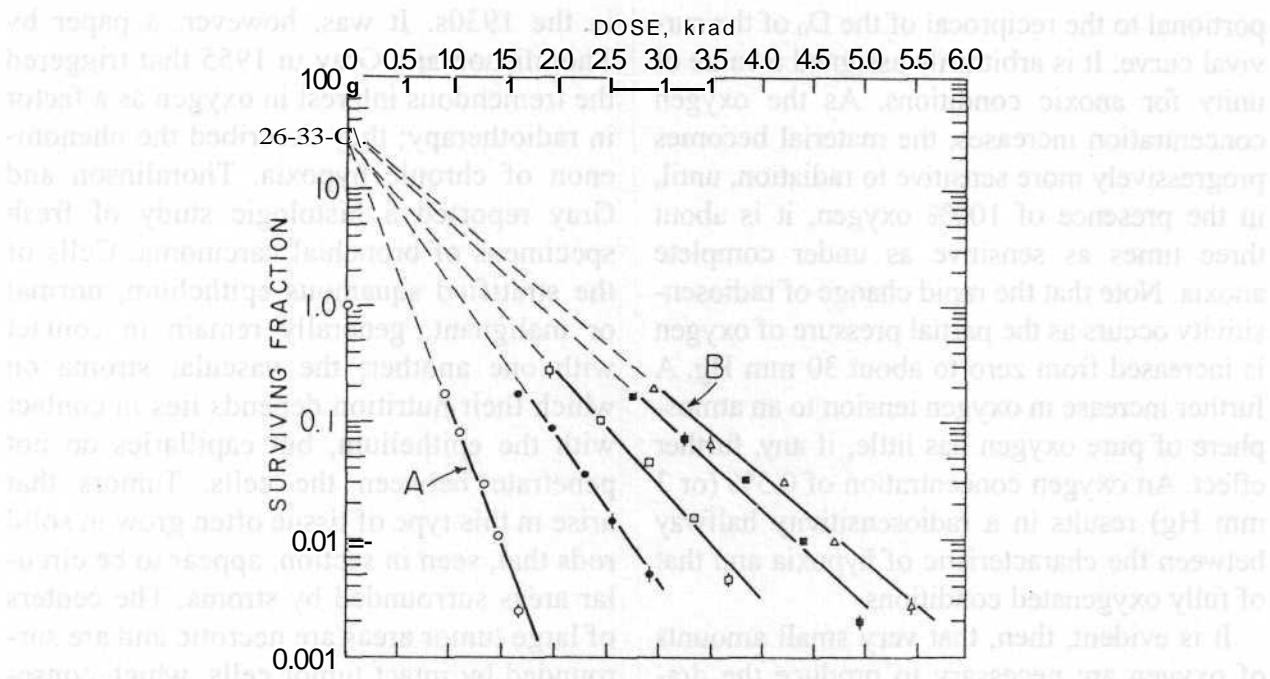


Figure 6.4. Survival curves for Chinese hamster cells exposed to x-rays in the presence of various oxygen concentrations. Open circles, air; closed circles, 2,200 ppm of oxygen or  $pO_2$  of 1.7 mm Hg; open squares, 355 ppm of oxygen or  $pO_2$  of 0.25 mm Hg; closed squares, 100 ppm of oxygen or  $pO_2$  of 0.075 mm Hg; open triangles, 10 ppm of oxygen or  $pO_2$  of 0.0076 mm Hg, which corresponded to the lowest level of hypoxia that could be obtained. (From Elkind MM, Swain RW, Alescio T, Sutton H, Moses WB: Oxygen, nitrogen, recovery and radiation therapy. In Cellular Radiation Biology, pp 442-461. Baltimore, Williams & Wilkins, 1965, with permission.)

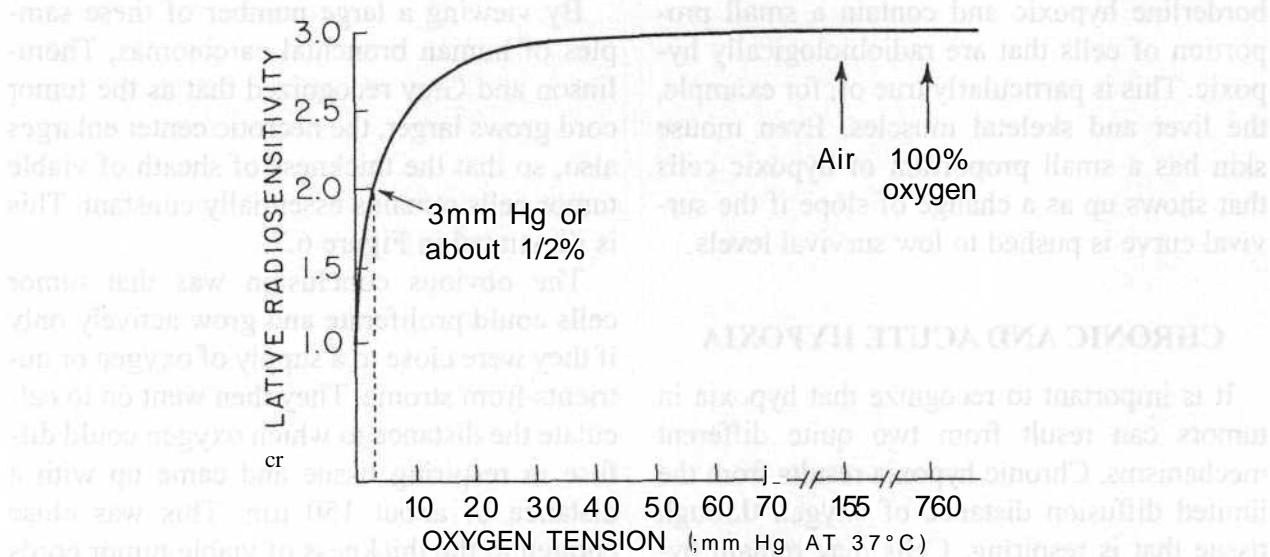


Figure 6.5. The dependence of radiosensitivity on oxygen concentration. If the radiosensitivity under anoxic conditions is arbitrarily assigned a value of unity, the radiosensitivity is about 3 under well-oxygenated conditions. Most of this change of sensitivity occurs as the oxygen concentration increases from 0 to 30 mm Hg. A further increase of oxygen content to that characteristic of air or even pure oxygen at high pressure has little further effect. A sensitivity halfway between anoxia and full oxygenation occurs for a  $pO_2$  of about 3 mm Hg, which corresponds to about 0.5%. This illustration is idealized and does not represent any specific experimental data. Experiments have been performed with yeast, bacteria, and mammalian cells in culture; the results conform to the general conclusions summarized here.

portional to the reciprocal of the Do of the survival curve. It is arbitrarily assigned a value of unity for anoxic conditions. As the oxygen concentration increases, the material becomes progressively more sensitive to radiation, until, in the presence of 100% oxygen, it is about three times as sensitive as under complete anoxia. Note that the rapid change of radiosensitivity occurs as the partial pressure of oxygen is increased from zero to about 30 mm Hg. A further increase in oxygen tension to an atmosphere of pure oxygen has little, if any, further effect. An oxygen concentration of 0.5% (or 3 mm Hg) results in a radiosensitivity halfway between the characteristic of hypoxia and that of fully oxygenated conditions.

It is evident, then, that very small amounts of oxygen are necessary to produce the dramatic and important oxygen effect observed with x-rays. Although it is usually considered that the oxygen tension of most normal tissues is similar to that of venous blood or lymph ( $20^40$  mm Hg), in fact oxygen probe measurements indicate that the oxygen tension between different tissues may vary over a wide range from 1 to 100 mm Hg. Many tissues are borderline hypoxic and contain a small proportion of cells that are radiobiologically hypoxic. This is particularly true of, for example, the liver and skeletal muscles. Even mouse skin has a small proportion of hypoxic cells that shows up as a change of slope if the survival curve is pushed to low survival levels.

### CHRONIC AND ACUTE HYPOXIA

It is important to recognize that hypoxia in tumors can result from two quite different mechanisms. Chronic hypoxia results from the limited diffusion distance of oxygen through tissue that is respiring. Cells may remain hypoxic for long periods of time. Acute hypoxia is a result of the temporary closing of a tumor blood vessel and is therefore transient.

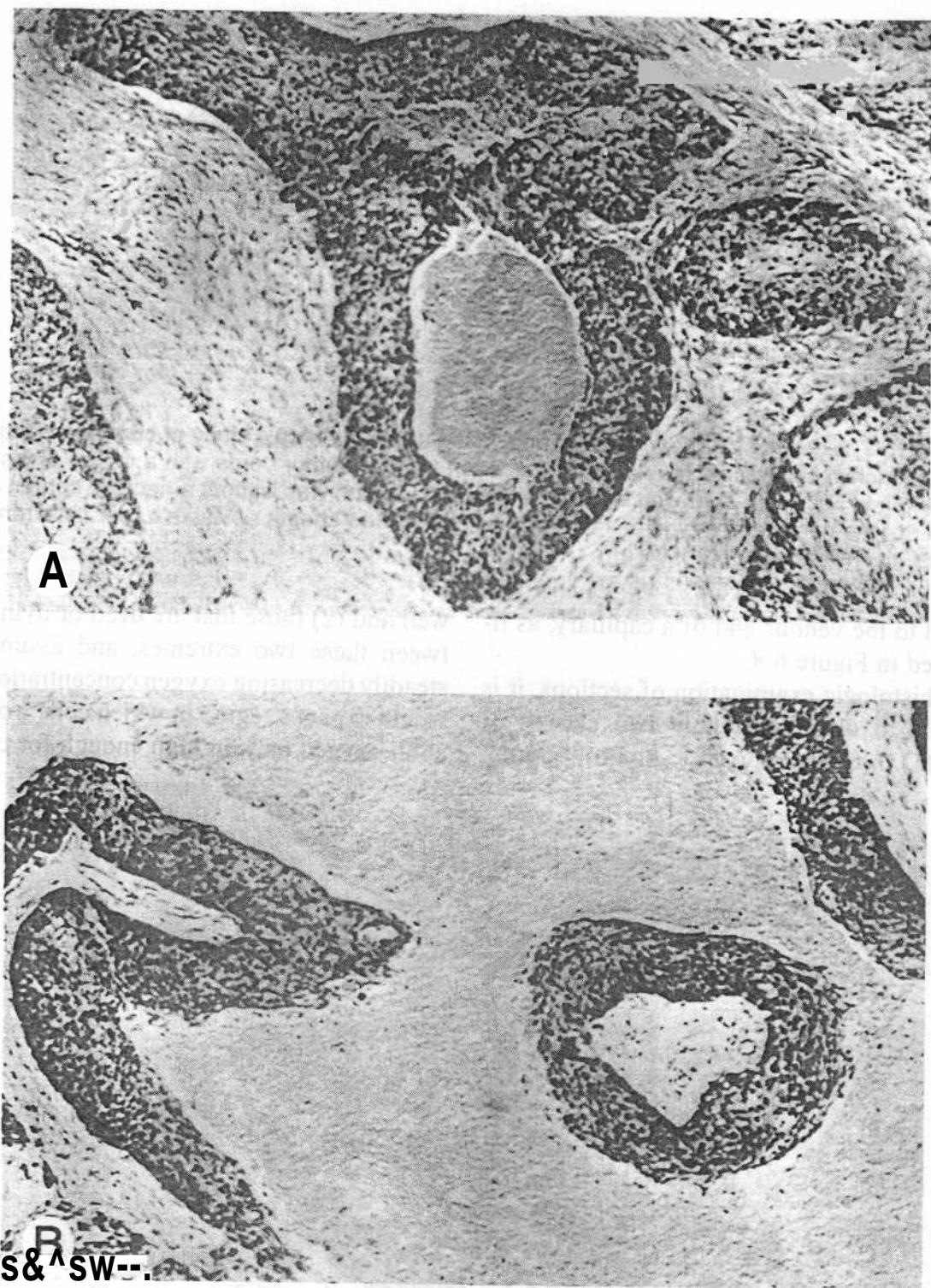
#### Chronic Hypoxia

Radiotherapists began to suspect that oxygen influences the radiosensitivity of tumors

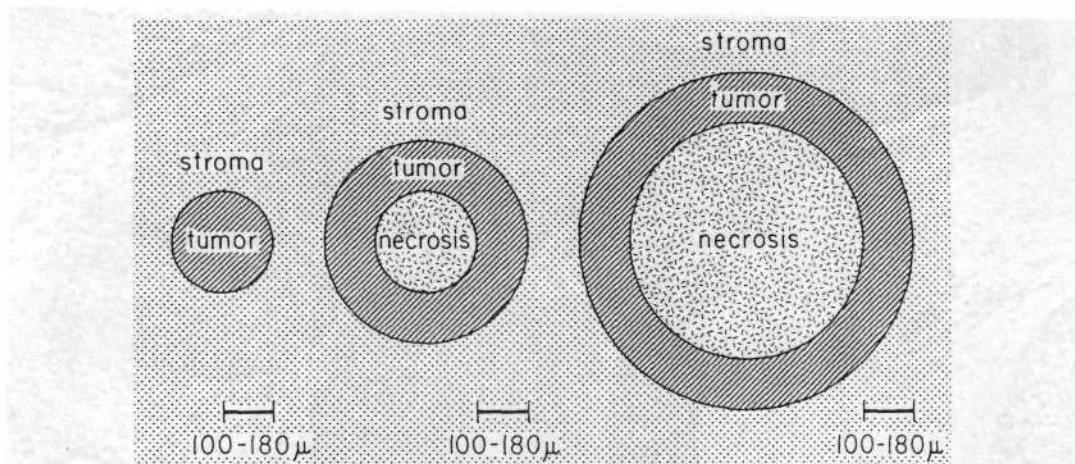
in the 1930s. It was, however, a paper by Thomlinson and Gray in 1955 that triggered the tremendous interest in oxygen as a factor in radiotherapy; they described the phenomenon of chronic hypoxia. Thomlinson and Gray reported a histologic study of fresh specimens of bronchial carcinoma. Cells of the stratified squamous epithelium, normal or malignant, generally remain in contact with one another; the vascular stroma on which their nutrition depends lies in contact with the epithelium, but capillaries do not penetrate between the cells. Tumors that arise in this type of tissue often grow in solid rods that, seen in section, appear to be circular areas surrounded by stroma. The centers of large tumor areas are necrotic and are surrounded by intact tumor cells, which consequently appear as rings. Figure 6.6A, reproduced from Thomlinson and Gray, shows a transverse section of tumor cord and is typical of areas of a tumor in which necrosis is not far advanced. Figure 6.6B shows large areas of necrosis separated from stroma by a narrow band of tumor cells about 100 (im wide).

By viewing a large number of these samples of human bronchial carcinomas, Thomlinson and Gray recognized that as the tumor cord grows larger, the necrotic center enlarges also, so that the thickness of sheath of viable tumor cells remains essentially constant. This is illustrated in Figure 6.7.

The obvious conclusion was that tumor cells could proliferate and grow actively only if they were close to a supply of oxygen or nutrients from stroma. They then went on to calculate the distance to which oxygen could diffuse in respiring tissue and came up with a distance of about 150 fim. This was close enough to the thickness of viable tumor cords on their histologic sections for them to conclude that oxygen depletion was the principal factor leading to the development of necrotic areas in tumors. Using more appropriate values of oxygen diffusion coefficients and consumption values, a better estimate of the distance oxygen can diffuse in respiring tissue is about 70 (im. This of course varies from the



**Figure 6.6.** Transverse sections of tumor cords surrounded by stroma from human carcinoma of the bronchus. A: A typical tumor area in which necrosis is not far advanced. B: Large areas of necrosis separated from the stroma by bands of tumor cells about 100 mm wide. (From Thominson RH, Grav LH: Br J Cancer 9:539-549, 1955, with permission.)

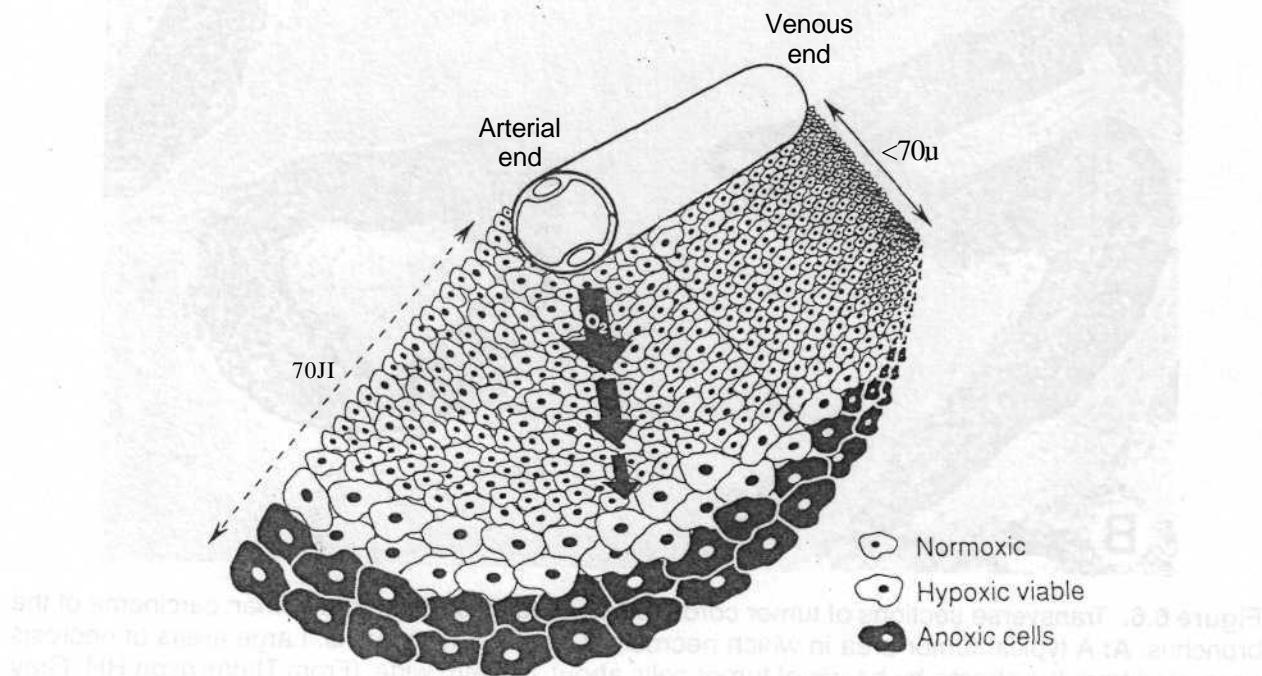


**Figure 6.7.** The conclusions reached by Thomlinson and Gray from a study of histologic sections of human bronchial carcinoma. No necrosis was seen in small tumor cords with a radius of less than about 160  $\mu$ m. No tumor cord with a radius exceeding 200  $\mu$ m was without a necrotic center. As the diameter of the necrotic area increased, the thickness of the sheath of viable tumor cells remained essentially constant at 100 to 180  $\mu$ m.

arterial to the venous end of a capillary, as illustrated in Figure 6.8.

By histologic examination of sections, it is possible to distinguish only two classes of cells: (1) those that appear to be proliferating

well and (2) those that are dead or dying. Between these two extremes, and assuming a steadily decreasing oxygen concentration, one would expect a region in which cells would be at an oxygen tension high enough for cells to



**Figure 6.8.** The diffusion of oxygen from a capillary through tumor tissue. The distance to which oxygen can diffuse is limited largely by the rapid rate at which it is metabolized by respiring tumor cells. For some distance from a capillary, cells are well oxygenated (white). At greater distances oxygen is depleted, and tumor cells become necrotic (black). Hypoxic tumor cells form a layer, perhaps one or two cells thick, in between (gray). In this region the oxygen concentration is high enough for the cells to be viable but low enough for them to be relatively protected from the effects of x-rays. These cells may limit the radiocurability of the tumor. The distance to which oxygen can diffuse is about 70  $\mu$ m at the arterial end of a capillary and less at the venous end.

be clonogenic but low enough to render the cells protected from the effect of ionizing radiation. Cells in this region would be relatively protected from a treatment with x-rays because of their low oxygen tension and could provide a focus for the subsequent regrowth of the tumor (Fig. 6.8). On the basis of these ideas, it was postulated that the presence of a relatively small proportion of hypoxic cells in tumors could limit the success of radiotherapy in some clinical situations.

These ideas about the role of oxygen in cell killing dominated the thinking of radiobiologists and radiotherapists in the late 1950s and early 1960s. A great deal of thought and effort was directed toward solving this problem. The solutions proposed included the use of high-pressure oxygen chambers and the development of novel radiation modalities, such as neutrons, negative  $\tau$ -mesons, and heavy charged ions.

### Acute Hypoxia

Regions of acute hypoxia develop in tumors as a result of the temporary closing or blockage of a particular blood vessel. If this blockage were permanent, the cells downstream, of course, would die eventually and would be of no further consequence. There is, however, good evidence that tumor blood vessels open and close in a random fashion so that different regions of the tumor become hypoxic intermittently. In fact, acute hypoxia results from blood-flow instability and is seldom a result of total stasis. At the moment at which a dose of radiation is delivered, a proportion of the tumor cells may be hypoxic, but if the radiation is delayed until a later time, a different group of cells may be hypoxic. The occurrence of acute hypoxia was postulated in the early 1980s by Brown and later demonstrated unequivocally in rodent tumors by Chaplin and his colleagues. Figure 6.9

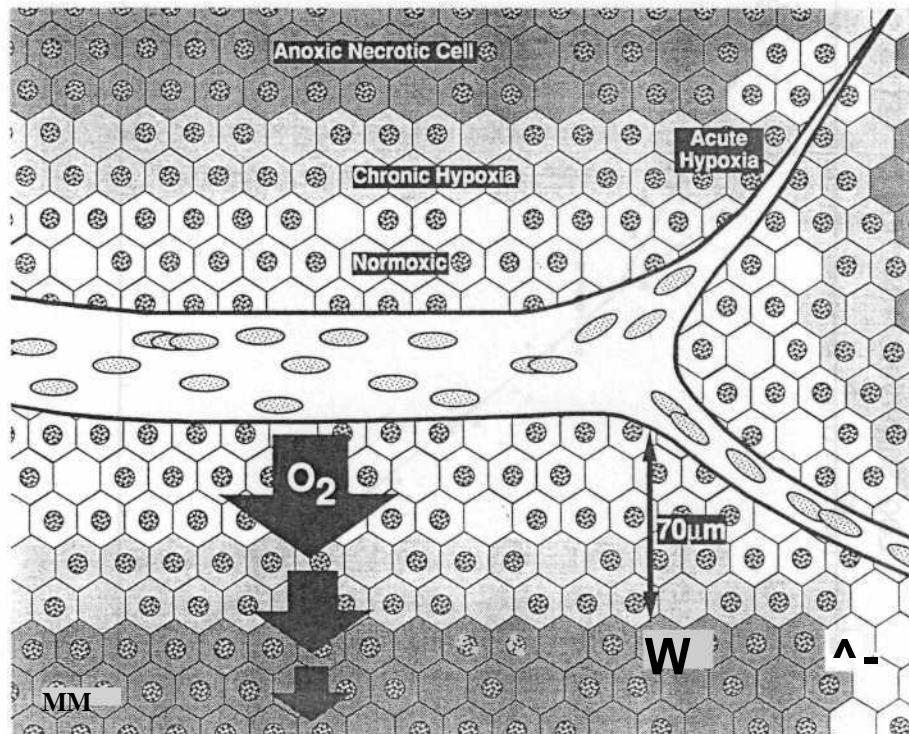


Figure 6.9. Diagram illustrating the difference between chronic and acute hypoxia. Chronic hypoxia results from the limited diffusion distance of oxygen in respiring tissue that is actively using up oxygen. Cells that become hypoxic in this way remain hypoxic for long periods of time until they die and become necrotic. Acute hypoxia results from the temporary closing of tumor blood vessels. The cells are intermittently hypoxic, because normoxia is restored each time the blood vessel opens up again. (Adapted from Brown JM: JNCI 82, 1990, with permission.)

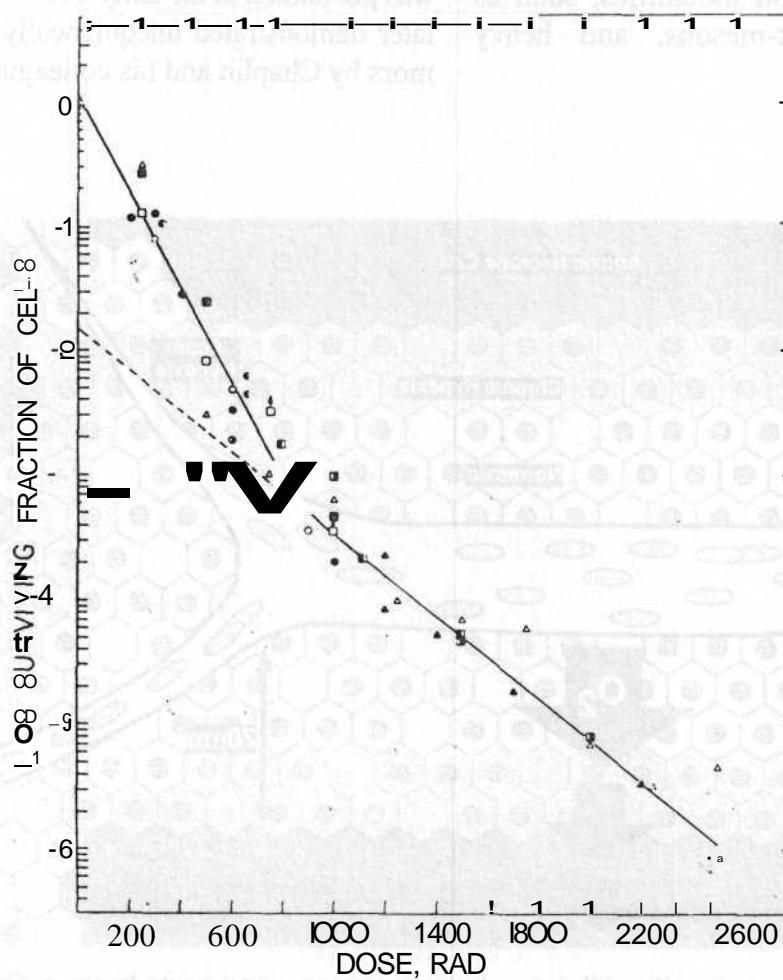
is an attempt to illustrate the way in which acute hypoxia occurs to explain the difference between acute and chronic hypoxia.

### THE FIRST EXPERIMENTAL DEMONSTRATION OF HYPOXIC CELLS IN A TUMOR

The dilution assay technique, described in Chapter 20, was used by Powers and Tolmach to investigate the radiation response of a solid subcutaneous lymphosarcoma in the mouse. Survival estimates were made for doses between 2 and 20 Gy (200 and 2,000 rad). The results are shown in Figure 6.10, in which the dose on a linear scale is plotted against the

fraction of surviving cells on a logarithmic scale.

The survival curve for this solid tumor clearly consists of two separate components. The first, up to a dose of about 9 Gy (900 rad), has a slope ( $D_0$ ) of 1.1 Gy (110 rad). The second has a shallower slope ( $D_0 = 2.6$  Gy, or 260 rad). This biphasic survival curve has a final slope about 2.5 times shallower than the initial portion, which strongly suggests that the tumor consists of two separate groups of cells, one oxygenated and the other hypoxic. If the shallow component of the curve is extrapolated backward to cut the surviving-fraction axis, it does so at a survival level of about 1%. From this it may be inferred that about



**Figure 6.10.** Fraction of surviving cells as a function of dose for a solid subcutaneous lymphosarcoma in the mouse irradiated *in vivo*. The first part of the curve has a slope ( $D_0$ ) of 1.1 Gy (110 rad); the second component of the curve has a shallower slope of 2.6 Gy (260 rad). (From Powers WE, Tolmach LJ: A multicomponent x-ray survival curve for mouse lymphosarcoma cells irradiated *in vivo*. Nature 197:710-711, 1963, with permission.)

1% of the clonogenic cells in the tumor were deficient in oxygen.

The response of this tumor to single doses of radiation of various sizes is explained readily on this basis. If 99% of the cells are well oxygenated and 1% are hypoxic, the response to lower doses is dominated by the killing of the well-oxygenated cells. For these doses the hypoxic cells are depopulated to a negligibly small extent. Once a dose of about 9 Gy (900 rad) is exceeded, however, the oxygenated compartment of the tumor is depopulated severely, and the response of the tumor is characteristic of the response of hypoxic cells. This biphasic survival curve was the first unequivocal demonstration that a solid tumor could contain cells sufficiently hypoxic to be protected from cell killing by x-rays but still clonogenic and capable of providing a focus for tumor regrowth.

### PROPORTION OF HYPOXIC CELLS IN VARIOUS ANIMAL TUMORS

Over the years many investigators have determined the fraction of hypoxic cells in a wide variety of tumors in experimental animals. The most satisfactory and most widely used method is to obtain paired survival curves (Fig. 6.11 A). The steepest curve relates to a fully oxygenated population of cells; the uppermost curve, to a population made up entirely of hypoxic cells. The intermediate curves refer to mixed populations of hypoxic and oxygenated cells. At low doses the survival curve for a mixed population closely follows that for the oxygenated population. At higher doses the number of surviving oxygenated cells is negligible compared with the number of anoxic cells, and consequently the curve representing the mixed population is parallel to (*i.e.*, has the same slope as) the curve for the hypoxic population. The fraction of hypoxic cells in the tumor determines the distance between the parallel terminal slopes of the dose-response curves, as shown in Figure 6.11 A. This fraction is identical to the ratio of the surviving cells from the partially hypoxic tumor to those from the entirely hypoxic tumor.

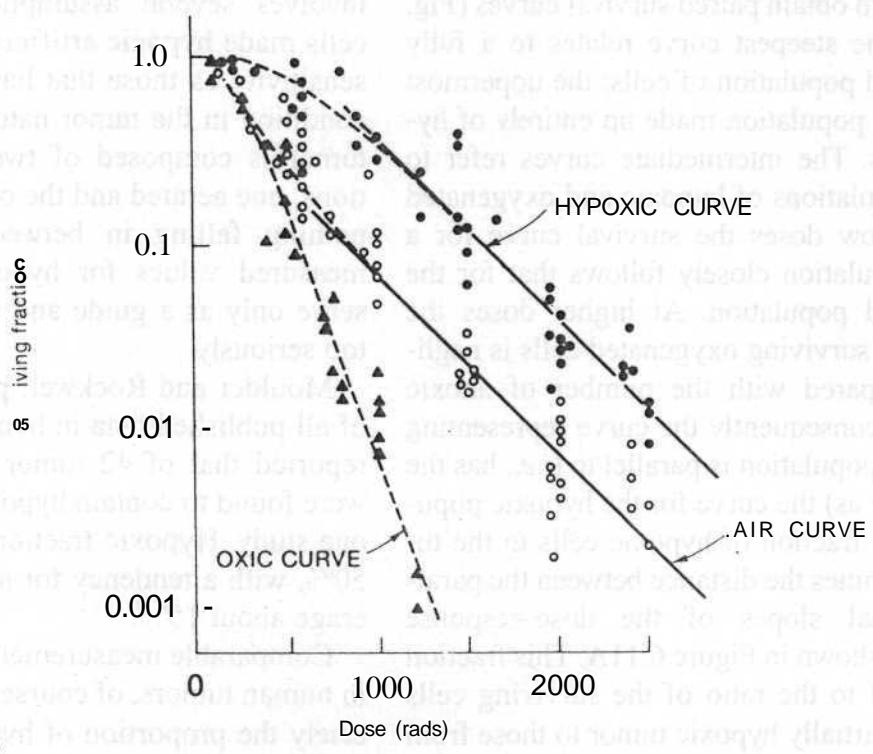
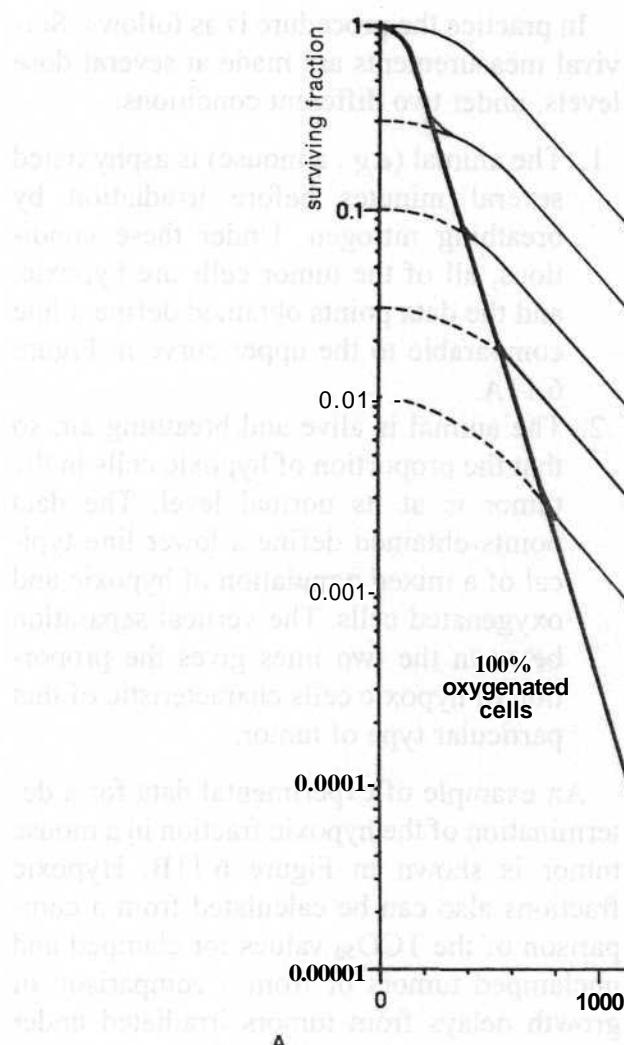
In practice the procedure is as follows: Survival measurements are made at several dose levels, under two different conditions:

1. The animal (*e.g.*, a mouse) is asphyxiated several minutes before irradiation by breathing nitrogen. Under these conditions, all of the tumor cells are hypoxic, and the data points obtained define a line comparable to the upper curve in Figure 6.11A.
2. The animal is alive and breathing air, so that the proportion of hypoxic cells in the tumor is at its normal level. The data points obtained define a lower line typical of a mixed population of hypoxic and oxygenated cells. The vertical separation between the two lines gives the proportion of hypoxic cells characteristic of that particular type of tumor.

An example of experimental data for a determination of the hypoxic fraction in a mouse tumor is shown in Figure 6.1 IB. Hypoxic fractions also can be calculated from a comparison of the TCD<sub>50</sub> values for clamped and undamped tumors or from a comparison of growth delays from tumors irradiated under these two conditions. Any of these methods involves several assumptions, notably that cells made hypoxic artificially have the same sensitivity as those that have respiration to this condition in the tumor naturally, and that the tumor is composed of two distinct populations, one aerated and the other hypoxic, with nothing falling in between. Consequently, measured values for hypoxic fractions can serve only as a guide and must not be taken too seriously.

Moulder and Rockwell published a survey of all published data in hypoxic fractions and reported that of 42 tumor types studied, 37 were found to contain hypoxic cells in at least one study. Hypoxic fractions range from 0 to 50%, with a tendency for many results to average about 15%.

Comparable measurements cannot be made in human tumors, of course, to determine precisely the proportion of hypoxic cells. By irradiating patients with multiple skin nodules



with and without large doses of the hypoxic cell sensitizer "misonidazole," Dische and Denekamp were able to estimate that the proportion of hypoxic cells was consistent with the 10 to 15% characteristic of many animal tumors.

### EVIDENCE FOR HYPOXIA IN HUMAN TUMORS

Although it is not possible to perform experiments in humans to unequivocally demonstrate that at least some tumors contain hypoxic cells, the circumstantial evidence is compelling:

- Analogy can be made with mouse tumors, in which hypoxia can be demonstrated unequivocally.
- Histologic appearance suggests the possibility of hypoxia.
- Binding of radioactive-labeled nitroimidazoles occurs.
- Oxygen-probe measurements are predictive.
- Pretreatment hemoglobin levels are a powerful prognostic factor in squamous carcinoma of the head and neck, carcinoma of the cervix, carcinoma of the bronchus, and transitional cell carcinoma of the bladder.

### OXYGEN PROBE MEASUREMENTS AS A PREDICTIVE ASSAY

**Oxygen probes**, that is, electrodes implanted directly into tumors to measure oxygen concentration by a polarographic technique, have a long and checkered history. Technical developments, however, have resulted in the Eppendorf probe, which has a very fast response time and can be moved quickly through a tumor under computer control to produce an oxygen profile.

Oxygen probes may be used in individual patients before treatment as a predictive assay to sort out those with tumors that are significantly hypoxic. A preliminary clinical trial in Germany suggested that local control in advanced carcinoma of the cervix, treated by radiotherapy, correlated with oxygen-probe measurements, indicating that this may be a useful predictive assay. Additional data showed, however, that hypoxia may be an indication of tumor aggression rather than resistance to x-rays in this case. This is discussed subsequently in this chapter.

### REOXYGENATION

Van Putten and Kallman determined the proportion of hypoxic cells in a transplantable sarcoma in the mouse. This tumor, which was

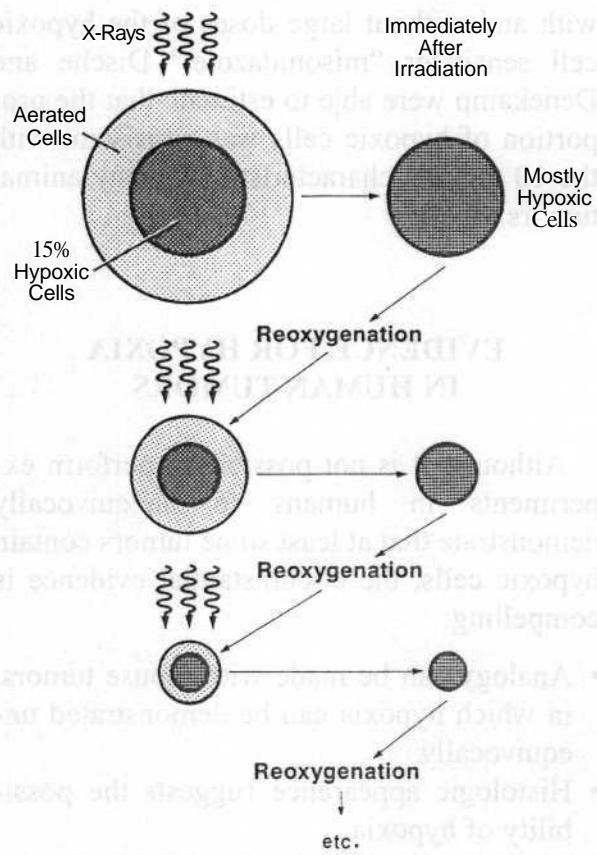
**Figure 6.11. A:** Theoretic survival curves for cell populations containing different fractions of hypoxic cells. The fraction of hypoxic cells in each population determines the distance between its survival curve and the curve for the completely hypoxic population. From the relative radiosensitivity at any dose level at which the survival curves are approximated by parallel lines, the fraction of hypoxic cells can be determined from the ratio of survival of the completely and partially hypoxic populations, as indicated by the vertical lines A-A, B-B, and so on. This illustration is based on the model proposed by Hewitt and Wilson. (From van Putten LM, Kallman RF: Oxygenation status of a transplantable tumor during fractionated radiotherapy. J Natl Cancer Inst 40:441-451, 1968, with permission.) **B:** The proportion of hypoxic cells in a mouse tumor. The biphasic curve labeled *air curve* represents data for cells from tumors irradiated in mice breathing air, which are therefore a mixture of aerated and hypoxic cells. The hypoxic curve is for cells irradiated in mice asphyxiated by nitrogen breathing or for cells irradiated *in vitro* in nitrogen, so that they are all hypoxic. The air curve is for cells irradiated *in vitro* in air. The proportion of hypoxic cells is the ratio of the air to hypoxic curves or the vertical separation between the curves, because surviving fraction is on a logarithmic scale. (Courtesy of Dr. Sara Rockwell; based on data of Moulder and Rockwell and of Rockwell and Kallman.)

of spontaneous origin, was transplanted from one generation of animals to the next by inoculating a known number of tumor cells subcutaneously. The tumor was allowed to grow for 2 weeks, by which time it had reached a size suitable for the experiment. The tumor was irradiated *in vivo* and then excised and made into a suspension of cells. The proportion of hypoxic cells was determined by the method described in Figure 6.11.

They found that for this mouse sarcoma the proportion of hypoxic cells in the untreated tumor was about 14%. The vital contribution made by van Putten and Kallman involved a determination of the proportion of hypoxic cells in this tumor after various fractionated radiation treatments. Groups of tumors were exposed to five daily doses of 1.9 Gy (190 rad), delivered from Monday through Friday, and the proportion of hypoxic cells was determined on the following Monday. The result was 18% hypoxic cells. In another experiment, four daily fractions were given, Monday through Thursday, and the proportion of hypoxic cells was measured on the following day, Friday. In this experiment the proportion of hypoxic cells was found to be 14%.

These experiments have far-reaching implications in radiotherapy. The fact that the proportion of hypoxic cells in the tumor is about the same at the end of a fractionated radiotherapy regimen as in the untreated tumor demonstrates that during the course of the treatment, cells move from the hypoxic to the well-oxygenated compartment of the tumor. If this were not the case, then the *proportion* of hypoxic cells would increase during the course of the fractionated treatment, because the radiation depopulates the aerated-cell compartment more than the hypoxic-cell compartment. This phenomenon, by which hypoxic cells become oxygenated after a dose of radiation, is termed reoxygenation. The oxygen status of cells in a tumor is not static; it is dynamic and constantly changing.

The process of reoxygenation is illustrated in Figure 6.12. A modest dose of x-rays to a mixed population of aerated and hypoxic cells results in significant killing of aerated cells,

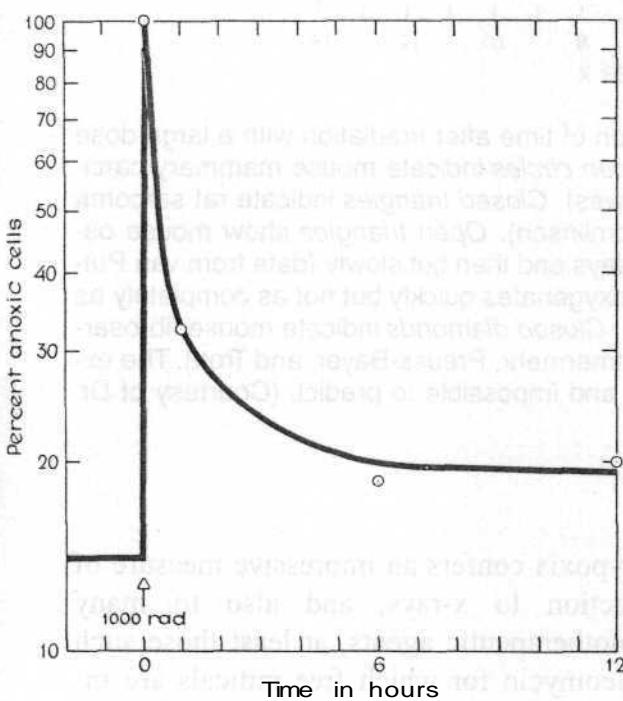


**Figure 6.12.** The process of reoxygenation. Tumors contain a mixture of aerated and hypoxic cells. A dose of x-rays kills a greater proportion of aerated than hypoxic cells, because they are more radiosensitive. Immediately after irradiation, most cells in the tumor are hypoxic. But the preirradiation pattern tends to return because of reoxygenation. If the radiation is given in a series of fractions separated in time sufficiently for reoxygenation to occur, the presence of hypoxic cells does not greatly influence the response of the tumor.

but little killing of those that are hypoxic. Consequently, the viable cell population immediately after irradiation is dominated by hypoxic cells. If sufficient time is allowed before the next radiation dose, the process of reoxygenation restores the proportion of hypoxic cells to about 15%. If this process is repeated many times, the tumor-cell population is depleted, despite the intransigence to killing by x-rays of the cells deficient in oxygen. In other words, if reoxygenation is efficient between dose fractions, the presence of hypoxic cells does have a significant effect on the outcome of a multifraction regimen.

### TIME SEQUENCE OF REOXYGENATION

The work of van Putten and Kallman indicated that, in their particular tumor system, the proportion of hypoxic cells had returned to its original pretreatment level by 24 hours after the delivery of a fractionated dosage schedule. Kallman and Bleehen reported experiments in which the proportion of hypoxic cells in the same transplantable mouse sarcoma was determined at various times after the delivery of a single dose of 10 Gy (1,000 rad). Their results are shown in Figure 6.13;



**Figure 6.13.** Percentage of hypoxic cells in a transplantable mouse sarcoma as a function of time after a dose of 10 Gy (1,000 rad) of x-rays. Immediately after irradiation, essentially 100% of the viable cells are hypoxic, because such a dose kills a large proportion of the aerated cells. In this tumor the process of reoxygenation is very rapid. By 6 hours, the percentage of hypoxic cells has fallen to a value close to the preirradiation level. (From Kallman RF, Bleehen NM: Post-irradiation cyclic radiosensitivity changes in tumors and normal tissues. In Brown DG, Cragle RG, Noonan JR (eds): Proceedings of the Symposium on Dose Rate in Mammalian Radiobiology, Oak Ridge, TN, 1968, pp 20.1-20.23. CONF-680410. Springfield, VA, 1968, with permission.)

the shape of the curve indicates that in this particular tumor the process of reoxygenation is very rapid indeed.

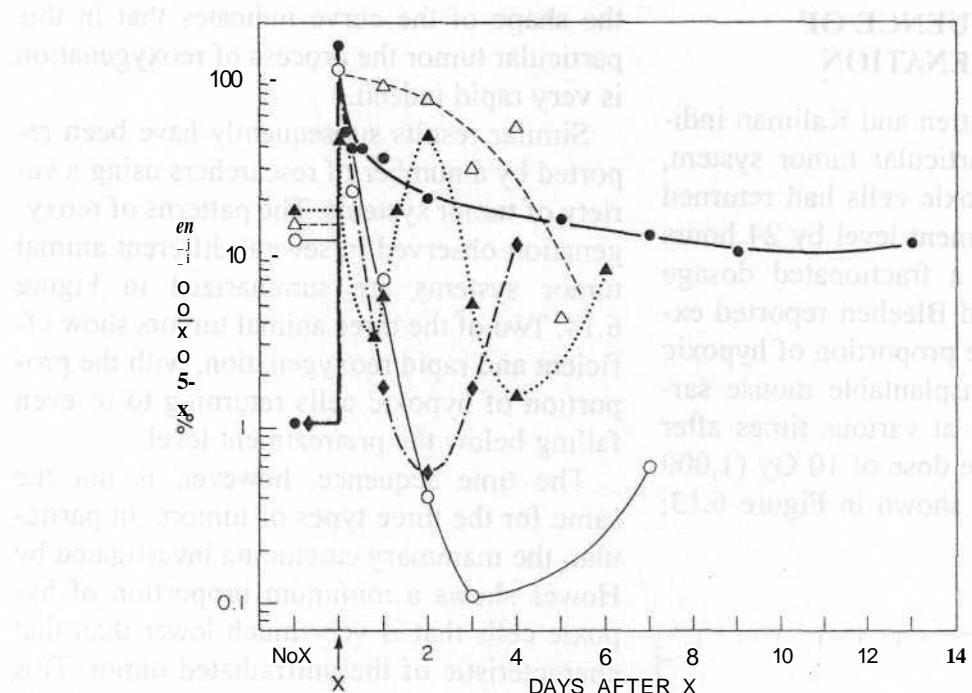
Similar results subsequently have been reported by a number of researchers using a variety of tumor systems. The patterns of reoxygenation observed in several different animal tumor systems are summarized in Figure 6.14. Two of the three animal tumors show efficient and rapid reoxygenation, with the proportion of hypoxic cells returning to or even falling below the pretreatment level.

The time sequence, however, is not the same for the three types of tumors. In particular, the mammary carcinoma investigated by Howes shows a minimum proportion of hypoxic cells that is very much lower than that characteristic of the unirradiated tumor. This occurs 3 days after the delivery of a single large dose of radiation. The only experimental tumor investigated so far that does not show any significant rapid reoxygenation is the osteosarcoma studied by van Putten, also illustrated in Figure 6.14.

### MECHANISM OF REOXYGENATION

In experimental animals, some tumors take several days to reoxygenate; in others the process appears to be complete within 1 hour or so. In a few tumors both fast and slow components to reoxygenation are evident. The differences of time scale reflect the different types of hypoxia that are being reversed, acute or chronic. In the long term, a restructuring or a revascularization of the tumor might be expected as the cells killed by the radiation are broken down and removed from the population. As the tumor shrinks in size, surviving cells that previously were beyond the range of oxygen diffusion find themselves closer to a blood supply and so reoxygenate. This slow component of reoxygenation, taking place over a period of days as the tumor shrinks, involves reoxygenation of cells that were *chronically* hypoxic.

By contrast, the first component of reoxygenation, which is complete within hours, is caused by the reoxygenation of acutely hy-



**Figure 6.14.** The proportion of hypoxic cells as a function of time after irradiation with a large dose for five transplanted tumors in experimental animals. Open circles indicate mouse mammary carcinoma that reoxygenates rapidly and well (data from Howes). Closed triangles indicate rat sarcoma that shows two waves of reoxygenation (data from Thomlinson). Open triangles show mouse osteosarcoma that does not reoxygenate at all for several days and then but slowly (data from van Putten). Closed circles indicate mouse fibrosarcoma that reoxygenates quickly but not as completely as the mammary carcinoma (data from Dorie and Kallman). Closed diamonds indicate mouse fibrosarcoma that reoxygenates quickly and well (data from Kummermehr, Preuss-Bayer, and Trott). The extent and rapidity of reoxygenation is extremely variable and impossible to predict. (Courtesy of Dr. Sara Rockwell.)

poxic cells. Those cells that are hypoxic at the time of irradiation because they are in a region in which a blood vessel is temporarily closed quickly reoxygenate when that vessel reopens.

### THE IMPORTANCE OF REOXYGENATION IN RADIOTHERAPY

The process of reoxygenation has important implications in practical radiotherapy. If human tumors do in fact reoxygenate as rapidly and efficiently as most of the animal tumors studied, then the use of a multifraction course of radiotherapy, extending over a long period of time, may well be all that is required to deal effectively with any hypoxic cells in human tumors.

Hypoxia confers an impressive measure of protection to x-rays, and also to many chemotherapeutic agents, at least those such as bleomycin for which free radicals are involved in the mechanism of cell killing.

The reoxygenation studies with C3H mouse mammary carcinoma, included in Figure 6.14, indicate that by 2 to 3 days after a dose of radiation, the proportion of hypoxic cells is actually lower than in untreated tumors. Consequently, it was predicted that several large doses of x-rays given at 48-hour intervals would virtually eliminate the problem of hypoxic cells in this tumor. Fowler and his colleagues showed that, for the cure of this tumor, the preferred x-ray schedule was five large doses in 9 days. These results suggest that x-irradiation can be an extremely effective form of therapy but ideally requires a

sharply optimal choice of fractionation pattern. Making this choice, however, demands a detailed knowledge of the time course of reoxygenation in the particular tumor to be irradiated. This information is available for only a few animal tumors and is impossible to obtain at present for humans. Indeed, in humans it is not known whether any or all tumors reoxygenate, although the evidence from the radiotherapy clinic that many tumors are eradicated with doses on the order of 60 Gy (6,000 rad) given in 30 treatments argues strongly in favor of reoxygenation, because the presence of a very small proportion of hypoxic cells would make "cures" unlikely at these dose levels. It is an attractive hypothesis that some of the human tumors that do not respond to conventional radiotherapy are those that do not reoxygenate quickly and efficiently.

### **HYPOXIA AND TUMOR PROGRESSION**

Evidence that low-oxygen conditions play an important role in malignant progression comes from studies of the correlation between tumor oxygenation and treatment outcome **in** patients, as well as from laboratory studies in cells and animals.

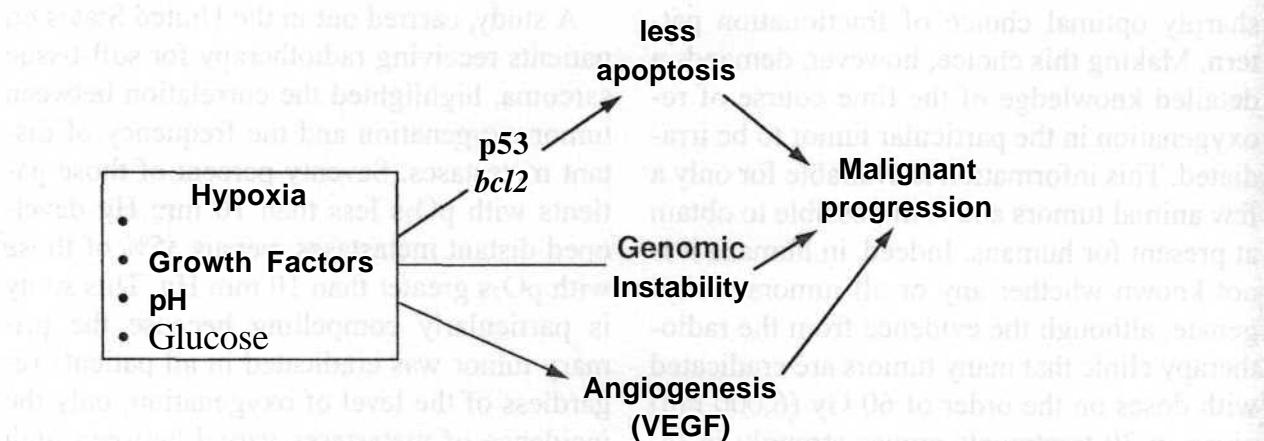
#### **Patient Studies**

Elsewhere in this chapter, reference is made to a clinical study in Germany that showed a correlation between local control in advanced carcinoma of the cervix, treated by radiotherapy, and oxygen-probe measurements. Specifically, patients in whom the probe measurements indicated pC<sup>2</sup>s greater than 10 mm Hg did better than those with pChs less than 10 mm Hg. Later studies, however, indicated a similar improvement in outcome for patients with better oxygenated tumors if the treatment was by surgery rather than radiotherapy. This suggests that the correct interpretation is that hypoxia is a general indicator of tumor aggression in these patients, rather than the initial view that hypoxia conferred radioresistance on some cells.

A study, carried out in the United States on patients receiving radiotherapy for soft-tissue sarcoma, highlighted the correlation between tumor oxygenation and the frequency of distant metastases. Seventy percent of those patients with pO<sub>2</sub>s less than 10 mm Hg developed distant metastases, versus 35% of those with pO<sub>2</sub>s greater than 10 mm Hg. This study is particularly compelling because the primary tumor was eradicated in all patients regardless of the level of oxygenation; only the incidence of metastases varied between high and low pCh values. This argues strongly that the level of tumor oxygenation influences the aggressiveness of the tumor.

#### **Laboratory Evidence**

There are at least three lines of evidence from experimental studies of a link between hypoxia and malignant progression. First, hypoxia destabilizes the genome by inducing gene amplification and increasing gene mutation. Although the mechanisms are far from clear, genomic instability results in the production of transformed cells with mutations that give them a survival advantage in adverse conditions. A second insight into how low-oxygen conditions may affect the aggressiveness of tumors is that hypoxia initiates apoptosis in oncogenically transformed cells and so selects for cells with reduced apoptotic sensitivity. Cells with a defective apoptotic pathway resulting from either inactivation of the p53 tumor-suppressor gene or overexpression of antiapoptotic genes such as bcl-2 have a survival advantage in a low-oxygen environment. The third consequence of hypoxia is the induction of proangiogenic stimulatory factors. Of those described to date, vascular endothelial growth factor is the one that possesses high specificity **in** stimulating endothelial cell proliferation and migration. It has yet to be demonstrated that there is a cause-and-effect relationship between increased angiogenic gene expression and metastasis. The conclusion of these experiments is illustrated in Figure 6.15.



**Figure 6.15.** Illustrating how hypoxia is linked with malignant progression. First, hypoxia destabilizes the genome leading to an increase in gene mutation. Second, hypoxia selects for cells with reduced apoptotic potential. Third, hypoxia induces proangiogenic factors. (Based on the ideas of Amato Giaccia.)

#### SUMMARY OF PERTINENT CONCLUSIONS

- The presence or absence of molecular oxygen dramatically influences the biologic effect of x-rays.
- The oxygen enhancement ratio (OER) is the ratio of doses without and with oxygen to produce the same biologic effect.
- The OER for x-rays is about 3 at high doses and is possibly lower (about 2) at doses below about 2 Gy (200 rad).
- OER decreases as linear energy transfer increases. The OER approaches unity (*i.e.*, no oxygen effect) at a linear energy transfer of about 160 keV/jim. For neutrons, the OER has an intermediate value of about 1.6.
- To produce its effect, oxygen must be present during the radiation exposure or at least during the lifetime of the free radicals.
- Oxygen "fixes" (*i.e.*, makes permanent) the damage produced by free radicals. In the absence of oxygen, damage produced by the indirect action may be repaired.
- Only a small quantity of oxygen is required for radiosensitization; 0.5% oxygen results in a sensitivity halfway between hypoxia and full oxygenation.
- There are two forms of hypoxia that are the consequence of different mechanisms: chronic hypoxia and acute hypoxia.
- Chronic hypoxia results from the limited diffusion range of oxygen through respiring tissue.
- Acute hypoxia is a result of the temporary closing of tumor blood vessels and is therefore transient.
- In either case there may be cells present during irradiation that are at a sufficiently low oxygen tension to be intransigent to killing by x-rays but high enough to be viable.
- Most transplantable tumors in animals have been shown to contain hypoxic cells that limit curability by single doses of x-rays. Hypoxic fractions vary from 0 to 50% but are frequently about 10 to 15%. Studies of hypoxic cell sensitizers indicate similar proportions in human tumor nodules.
- There is good evidence that human tumors contain hypoxic cells, also. This evidence includes histologic appearance, oxygen-probe measurements, the binding of radioactive ni-

troimidazoles, and the importance of pretreatment hemoglobin levels as a prognostic factor for several types of malignancies.

- Oxygen probes with a fast response time, implanted in a tumor and moving quickly under computer control, may be used to obtain the oxygen profile of a tumor. One clinical trial suggests a correlation between median pO<sub>2</sub> values and local control in advanced carcinoma of the cervix treated by radiotherapy. This is discussed further in Chapter 15.
- Reoxygenation is the process by which cells that are hypoxic at the time of irradiation become oxygenated afterward.
- The extent of reoxygenation and the rapidity with which it occurs vary widely for different experimental animal tumors.
- If reoxygenation is rapid and complete, hypoxic cells have little influence on the outcome of a fractionated radiation schedule.
- The "fast" component of reoxygenation is caused by the reoxygenation of acutely hypoxic cells; as tumor blood vessels open and close, the slow component is caused by the reoxygenation of *chronically* hypoxic cells as the tumor shrinks.
- Reoxygenation cannot be measured in human tumors, but presumably it occurs, at least in those tumors controlled by conventional fractionated radiotherapy.
- There is clinical evidence that hypoxia may play an important role in malignant progression:
  1. Clinical studies show that local control in advanced carcinoma of the cervix, treated by radiotherapy or surgery, was superior in patients in whom oxygen probe measurements indicated pO<sub>2</sub>S greater than 10 mm Hg compared with pO<sub>2</sub>S less than 10 mm Hg.
  2. Other clinical studies of patients receiving radiotherapy for soft-tissue sarcoma showed a frequency of distant metastases of 70% of patients with pO<sub>2</sub>S less than 10 mm Hg versus 35% in those with pO<sub>2</sub>S more than 10 mm Hg.
- There is laboratory evidence of a correlation between hypoxia and malignant progression:
  1. Hypoxia destabilizes the genome, resulting in mutations that give cells a survival advantage in adverse conditions.
  2. Hypoxia selects for cells with reduced apoptotic activity. This may result from inactivation of the p53 tumor-suppressor gene or overexpression of antiapoptotic genes such as bcl-2. Such cells have a survival advantage in a low-oxygen environment.
- Evidence for hypoxia in human tumors is really outside the scope of a book on radiobiology, but for completeness, the reasons for thinking that some human tumors contain a significant proportion of hypoxic cells are listed here:
  1. Local control by radiotherapy, but not survival (because of distant metastasis), correlates closely with hemoglobin levels. This has been shown to be true for squamous cell carcinoma of the head and neck, cervix, and bronchus, as well as transitional cell carcinoma of the bladder.
  2. The histologic appearance of many human tumors suggests regions of hypoxia by analogy with animal tumors, in which the proportion of hypoxic cells can be measured.
  3. Measurements with oxygen probes indicate regions of hypoxia in some human tumors; local control correlates with pO<sub>2</sub> levels.
  4. Labeled nitroimidazoles are deposited near necrotic areas in some human tumors. This requires bioreduction and therefore implies hypoxia (Chapter 10).
  5. Hyperbaric oxygen and radiosensitizers such as misonidazole improve the results of radiotherapy in a few cases, particularly if a limited (suboptimal) number of dose fractions is used.

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## THE DEPOSITION OF RADICAL ENERGY

The deposition of radical energy in biological systems is often referred to as the "radiation dose." The term "dose" is derived from the Latin word "dos," which means "portion" or "share." In the field of radiation biology, the term "dose" refers to the amount of energy deposited in a unit mass of tissue. The unit of dose is the gray (Gy), which is defined as the absorption of 1 Joule of energy per kilogram of tissue. The gray is a relatively large unit of dose, and it is often used in conjunction with smaller units such as the rad (1 rad = 10 Gy) or the sievert (1 sievert = 100 Gy). The sievert is a unit of dose that takes into account the type of radiation and its biological effect. For example, alpha radiation is more biologically effective than beta radiation, so a given dose of alpha radiation would result in a higher sievert value than a similar dose of beta radiation.

## Linear Energy Transfer and Relative Biological Effectiveness

THE DEPOSITION OF RADIANT ENERGY  
 LINEAR ENERGY TRANSFER  
 RELATIVE BIOLOGIC EFFECTIVENESS  
 RELATIVE BIOLOGIC EFFECTIVENESS  
     AND FRACTIONATED DOSES  
 RELATIVE BIOLOGIC EFFECTIVENESS  
     FOR DIFFERENT CELLS AND TISSUES  
 RELATIVE BIOLOGIC EFFECTIVENESS AS  
     A FUNCTION OF LET

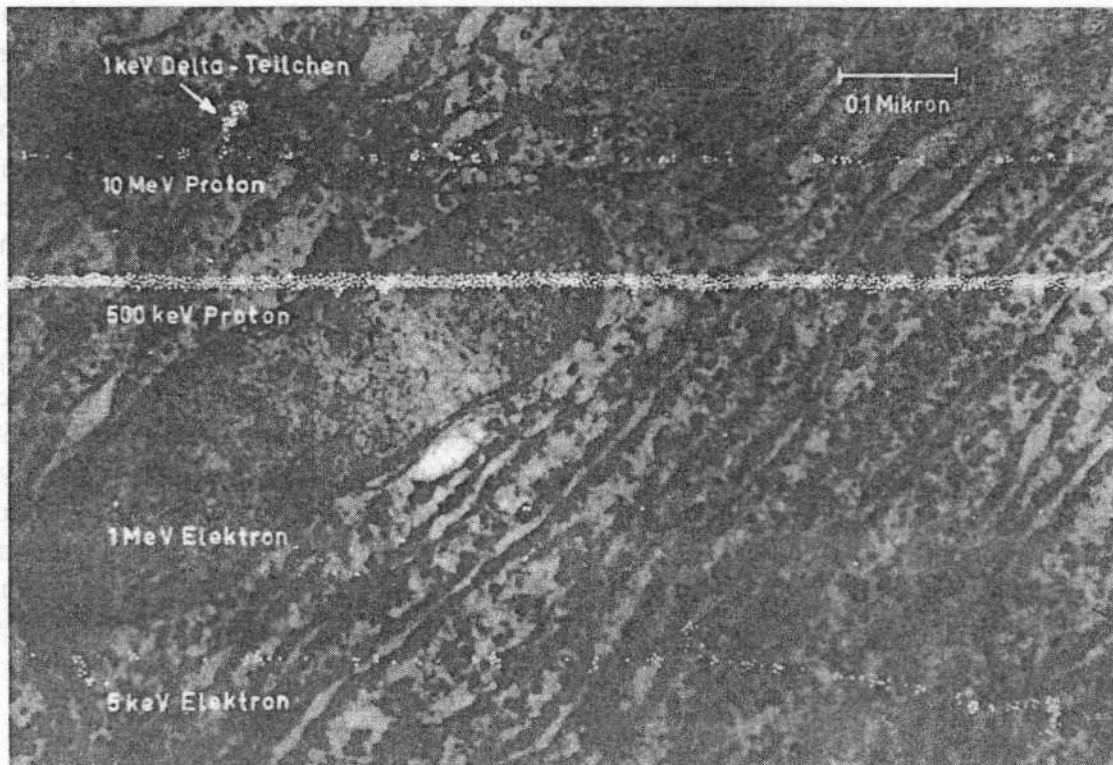
THE OPTIMAL LINEAR ENERGY  
     TRANSFER  
 FACTORS THAT DETERMINE RELATIVE  
     BIOLOGIC EFFECTIVENESS  
 THE OXYGEN EFFECT AND LINEAR  
     ENERGY TRANSFER  
 RADIATION WEIGHTING FACTOR  
 SUMMARY OF PERTINENT CONCLUSIONS

### **THE DEPOSITION OF RADIANT ENERGY**

If radiation is absorbed in biologic material, ionizations and excitations occur that are not distributed at random but tend to be localized along the tracks of individual charged particles in a pattern that depends on the type of radiation involved. For example, photons of x-rays give rise to fast electrons, particles carrying unit electric charge and having very small mass; neutrons, on the other hand, give rise to recoil protons, particles again carrying unit electric charge but having mass nearly 2,000 times greater than that of the electron.  $\alpha$ -Particles carry two electric charges on a particle four times as heavy as a proton. The charge-to-mass ratio for  $\alpha$ -particles therefore differs from that for electrons by a factor of about 8,000.

As a result, the spatial distribution of the ionizing events produced by different particles varies enormously. This is illustrated in

Figure 7.1. The background is an electron micrograph of a human liver cell. The white dots generated by a computer simulate ionizing events. The lowest track represents a low-energy electron, such as might be set in motion by a photon of diagnostic x-rays. The primary events are well separated in space, and for this reason x-rays are said to be **sparsely ionizing**. The second track from the bottom represents an electron set in motion by a photon of cobalt-60  $\gamma$ -rays, which is even more sparsely ionizing. For a given particle type, the density of ionization decreases as the energy goes up. The third track from the bottom represents a proton that might be set in motion by a fission spectrum neutron from a nuclear reactor; a dense column of ionization is produced, so the radiation is referred to as **densely ionizing**. The uppermost track refers to a 10-MeV proton, such as may be set in motion by a high-energy neutron used for radiotherapy. The track is intermediate in ionization density.



**Figure 7.1.** Variation of ionization density associated with different types of radiation. The background is an electron micrograph of a human cell. The white dots represent ionizations. **Top to bottom:** A 10-MeV proton, typical of the recoil protons produced by high-energy neutrons used for radiotherapy. The track is intermediate in ionization density. Also shown is a secondary 1-keV  $\gamma$ -ray, an electron set in motion by the proton. A 500-keV proton, produced by lower-energy neutrons (e.g., from fission spectrum) or by higher-energy neutrons after multiple collisions. The ionizations form a dense column along the track of the particle. A 1-MeV electron, produced, for example, by photons of cobalt-60  $\gamma$ -rays. This particle is very sparsely ionizing. A 5-keV electron, typical of secondary electrons produced by x-rays of diagnostic quality. This particle is also sparsely ionizing but a little denser than the higher-energy electron. (Courtesy of Dr. Albrecht Kellerer.)

## LINEAR ENERGY TRANSFER

**Linear energy transfer (LET)** is the energy transferred per unit length of the track. The special unit usually used for this quantity is kiloelectron volt per micrometer (keV/ $\mu\text{m}$ ) of unit density material. The International Commission on Radiological Units in 1962 defined this quantity as follows:

The linear energy transfer ( $L$ ) of charged particles in medium is the quotient of  $dE/dl$ , where  $dE$  is the average energy locally imparted to the medium by a charged particle of specified energy in traversing a distance of  $dl$ .

That is,

$$L = dE/dl$$

LET can be only an average quantity because, at the microscopic level, the energy per unit length of track varies over such a wide range that the average has very little meaning. This can be illustrated by the story of a Martian visitor to Earth who arrives knowing that Earth is inhabited by living creatures with an average mass of 1 g. Not only is this information of very little use, but it also may be positively misleading, particularly if the first animal that the Martian encounters is an elephant. An average has little meaning if individual variation is great.

The situation for LET is further complicated by the fact that it is possible to calculate an average in many different ways. The most

**LET = Average energy deposited per unit length of track (keV/μm)**

Track Average |oooooooooooo|

Energy Average |oooo|oooo|oooo|oooo|

**Figure 7.2.** Linear energy transfer (LET) is the average energy deposited per unit length of track. The track average is calculated by dividing the track into equal lengths and averaging the energy deposited in each length. The energy average is calculated by dividing the track into equal energy intervals and averaging the lengths of the tracks that contain this amount of energy. The method of averaging makes little difference for x-rays or for monoenergetic charged particles, but the track average and energy average are different for neutrons.

commonly used method is to calculate the **track average**, which is obtained by dividing the track into equal lengths, calculating the energy deposited in each length, and finding the mean. This is illustrated in Figure 7.2. The **energy average** is obtained by dividing the track into equal energy increments and averaging the lengths of track over which these energy increments are deposited.

In the case of either x-rays or monoenergetic charged particles, the two methods of averaging yield similar results. In the case of 14-MeV neutrons, by contrast, the track average LET is about 12 keV/μm and the energy average LET is about 75 keV/μm. The biologic properties of neutrons tend to correlate best with the energy average.

As a result of these considerations, LET is a quantity condemned by the purists as worse

than useless, because it can in some circumstances be very misleading. It is, however, useful as a simple and naive way to indicate the quality of different types of radiation. Typical LET values for commonly used radiations are listed in Table 7.1. Note that for a given type of charged particle, the higher the energy, the lower the LET and therefore the lower its biologic effectiveness. At first sight this may be counterintuitive. For example, γ-rays and x-rays both give rise to fast secondary electrons; therefore 1.1-MV cobalt-60 γ-rays have lower LETs than 250-kV x-rays and are less effective biologically by about 10%. By the same token, 150-MeV protons have lower LETs than 10-MeV protons and therefore are slightly less effective biologically.

### RELATIVE BIOLOGIC EFFECTIVENESS

The amount or quantity of radiation is expressed in terms of the **absorbed dose**, a physical quantity with the unit of gray or rad. Dose is a measure of the energy absorbed per unit mass of tissue. Equal doses of different types of radiation do not, however, produce equal biologic effects. One gray of neutrons produces a greater biologic effect than 1 Gy of x-rays. The key to the difference lies in the pattern of energy deposition at the microscopic level.

In comparing different radiations it is customary to use x-rays as the standard. The formal definition of **relative biologic effectiveness** (RBE) is as follows:

**TABLE 7.1. Typical Linear Energy Transfer Values**

Radiation	Linear Energy Transfer, KeV/μm	Track Avg.	Energy Avg.
Cobalt-60 γ-rays	0.2		
250-kV x-rays	2.0		
10-MeV protons	4.7		
150-MeV protons	0.5		
14-MeV neutrons	12		100
2.5-MeV α-particles		166	
2-GeV Fe ions		1,000	

The RBE of some test radiation ( $\bullet$ ) compared with x-rays is defined by the ratio  $D_{250}/D_r$ , where  $D_{250}$  and  $D_r$  are, respectively, the doses of x-rays and the test radiation required for equal biological effect.

To measure the RBE of some test radiation, one first chooses a biologic system in which the effect of radiations may be scored quantitatively. To illustrate the process involved, we discuss a specific example. Suppose we are measuring the RBE of fast neutrons compared with 250-kV x-rays, using the lethality of plant seedlings as a test system. Groups of plants are exposed to various doses of x-rays; parallel groups are exposed to a range of neutron doses. At the end of the period of observation, it is possible to calculate the doses of x-rays and then of neutrons that result in the death of half of the plants in a group. This quantity is known as the  $LD_{50}$ , the mean lethal dose. Suppose that for x-rays the  $LD_{50}$  turns out to be 6 Gy (600 rad) and that for neutrons

the corresponding quantity is 4 Gy (400 rad). The RBE of neutrons compared with x-rays is then simply the ratio 6:4, or 1.5.

The study of RBE is relatively straightforward so long as a test system with a single, unequivocal endpoint is used. It becomes more complicated if, instead, a test system such as the response of mammalian cells in culture is chosen. Figure 7.3A shows survival curves obtained if mammalian cells in cultures are exposed to a range of doses of, on the one hand, fast neutrons and, on the other hand, 250-kV x-rays. The RBE may now be calculated from these survival curves as the ratio of doses that produce the same biologic effect. If the endpoint chosen for comparison is the dose required to produce a surviving fraction of 0.01, then the dose of neutrons necessary is 6.6 Gy (660 rad); the corresponding dose of x-rays is 10 Gy (1,000 rad). The RBE, then, is the quotient of 10/6.6, or 1.5. If the comparison is made at a surviving fraction of 0.6, however,

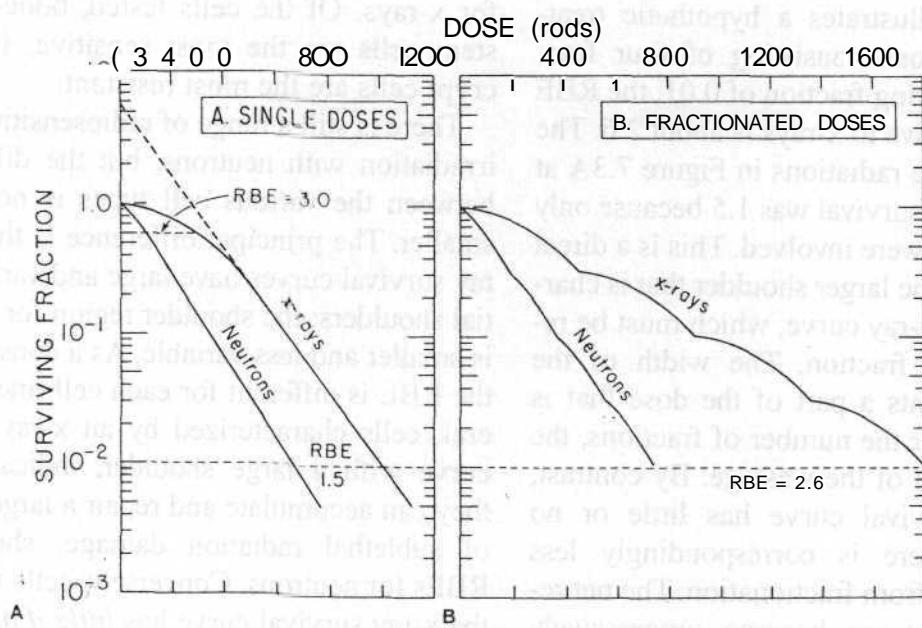


Figure 7.3. Typical survival curves for mammalian cells exposed to x-rays and fast neutrons. A. Single doses. The survival curve for x-rays has a large initial shoulder; for fast neutrons the initial shoulder is smaller and the final slope steeper. Because the survival curves have different shapes, the relative biologic effectiveness (RBE) does not have a unique value but varies with dose, getting larger as the size of the dose is reduced. B. Fractionated doses. The effect of giving doses of x-rays or fast neutrons in four equal fractions to produce the same level of survival as in A. The shoulder of the survival curves is reexpressed after each dose fraction; the fact that the shoulder is larger for x-rays than for neutrons results in an enlarged RBE for fractionated treatments.

the neutron dose required is only 1 Gy (100 rad), and the corresponding x-ray dose is 3 Gy (300 rad). The resultant RBE is 3:1, or 3.0. Because the x-ray and neutron survival curves have different shapes, the x-ray survival curve having an initial shoulder and the neutron curve being an exponential function of dose, the resultant RBE depends on the level of biologic damage (and therefore the dose) chosen. The RBE generally increases as the dose is decreased, reaching a limiting value that is the ratio of the initial slopes of the x-ray and neutron survival curves.

### RELATIVE BIOLOGIC EFFECTIVENESS AND FRACTIONATED DOSES

Because the RBE of more densely ionizing radiations, such as neutrons, varies with the dose per fraction, the RBE for a fractionated regimen with neutrons is greater than for a single exposure, because a fractionated schedule consists of a number of small doses and the RBE is large for small doses.

Figure 7.3B illustrates a hypothetic treatment with neutrons consisting of four fractions. For a surviving fraction of 0.01, the RBE for neutrons relative to x-rays is about 2.6. The RBE for the same radiations in Figure 7.3A at the same level of survival was 1.5 because only single exposures were involved. This is a direct consequence of the larger shoulder that is characteristic of the x-ray curve, which must be repeated for each fraction. The width of the shoulder represents a part of the dose that is wasted; the larger the number of fractions, the greater the extent of the wastage. By contrast, the neutron survival curve has little or no shoulder, so there is correspondingly less wastage of dose from fractionation. The net result is that neutrons become progressively more efficient than x-rays as the dose per fraction is reduced and the number of fractions is increased. The same is true, of course, for exposure to continuous low dose-rate irradiation. The neutron RBE is larger at a low dose rate than for an acute exposure, because the effectiveness of neutrons decreases with dose rate to

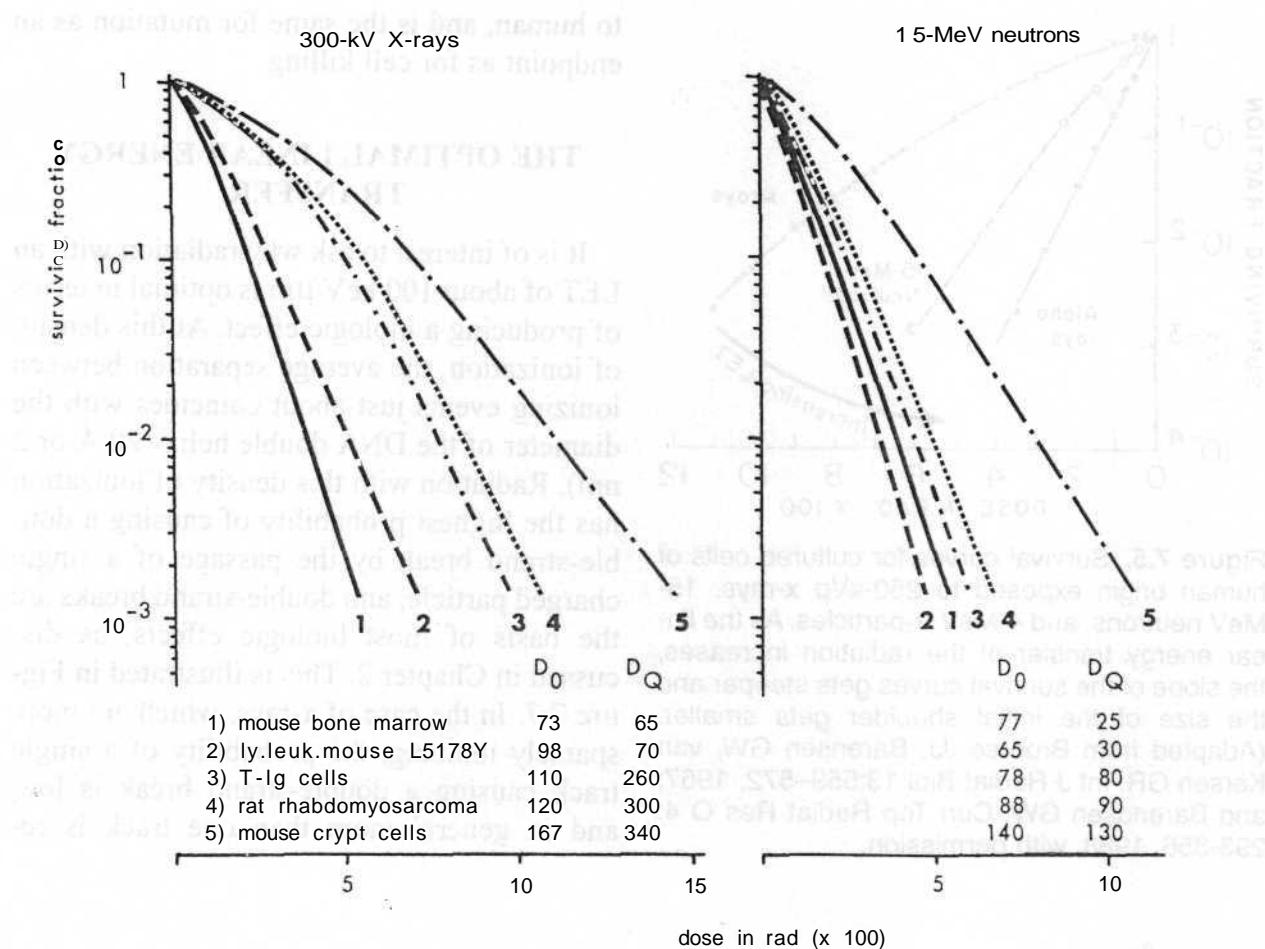
a much smaller extent than is the case for x- or y-rays. Indeed, for low-energy neutrons there is no loss of effectiveness.

### RELATIVE BIOLOGIC EFFECTIVENESS FOR DIFFERENT CELLS AND TISSUES

Even for a given total dose or dose per fraction, the RBE varies greatly according to the tissue or endpoint studied. Broerse and Barendsen and their colleagues in the Netherlands have obtained survival curves for a number of different cell lines exposed to either neutrons or x-rays. A summary of their data is shown in Figure 7.4, which illustrates the differences in intrinsic radiosensitivity among the various types of cells. In this figure survival curves are presented for mouse bone-marrow stem cells, mouse lymphocytic leukemia cells, cultured cells of human kidney origin, rat rhabdomyosarcoma cells, and mouse intestinal crypt cells. These curves demonstrate clearly that different cells exhibit a considerable spectrum of radiosensitivities for x-rays. Of the cells tested, bone-marrow stem cells are the most sensitive; intestinal crypt cells are the most resistant.

There is still a range of radiosensitivities for irradiation with neutrons, but the differences between the various cell types is now much smaller. The principal difference is that the x-ray survival curves have large and variable initial shoulders; the shoulder region for neutrons is smaller and less variable. As a consequence, the RBE is different for each cell line. In general, cells characterized by an x-ray survival curve with a large shoulder, indicating that they can accumulate and repair a large amount of sublethal radiation damage, show large RBEs for neutrons. Conversely, cells for which the x-ray survival curve has *little if any shoulder* exhibit small neutron RBE values.

In Figure 7.4 the crypt cells of the mouse jejunum have the largest shoulder to their x-ray survival curve; they also result in the highest neutron RBE. At the other end of the scale, colony-forming units in the bone marrow are characterized by a **survival** curve that is close



**Figure 7.4.** Survival curves for various types of clonogenic mammalian cells irradiated with 300-kV x-rays or 15-MeV  $d^+ \wedge T$  neutrons: curve 1, mouse hematopoietic stem cells; curve 2, mouse lymphocytic leukemia cells L5178Y; curve 3, T1 cultured cells of human kidney origin; curve 4, rat rhabdomyosarcoma cells; curve 5, mouse intestinal crypt stem cells. Note that the variation in radiosensitivity among different cell lines is markedly less for neutrons than for x-rays (From Broerse JJ, Barendsen GW: Relative biological effectiveness of fast neutrons for effects on normal tissues. Curr Top Radiat Res Q 8:305-350, 1973, with permission.)

to an exponential function of dose, with little if any shoulder. The RBE for neutrons is likewise smallest for this biologic system.

### RELATIVE BIOLOGIC EFFECTIVENESS AS A FUNCTION OF LINEAR ENERGY TRANSFER

Figure 7.5 illustrates the survival curves obtained for x-rays, 15-MeV neutrons, and  $\alpha$ -particles. As the LET increases from about 2 keV/jim for x-rays up to 150 keV/jim for  $\alpha$ -particles, the survival curve changes in two important respects. First, the survival curve becomes steeper. Second, the shoulder of the curve be-

comes progressively smaller as the LET increases. A more common way to represent these data is to plot the RBE as a function of LET (Fig. 7.6). As the LET increases, the RBE increases slowly at first, and then more rapidly as the LET increases beyond 10 keV/jim. Between 10, and 100 keV/jim, the RBE increases rapidly with increasing LET and in fact reaches a maximum at about 100 keV/jim. Beyond this value for the LET, the RBE again falls to lower values. This is an important effect and is explained in more detail in the next section.

The LET at which the RBE reaches a peak is much the same (about 100 keV/jim) for a wide range of mammalian cells, from mouse

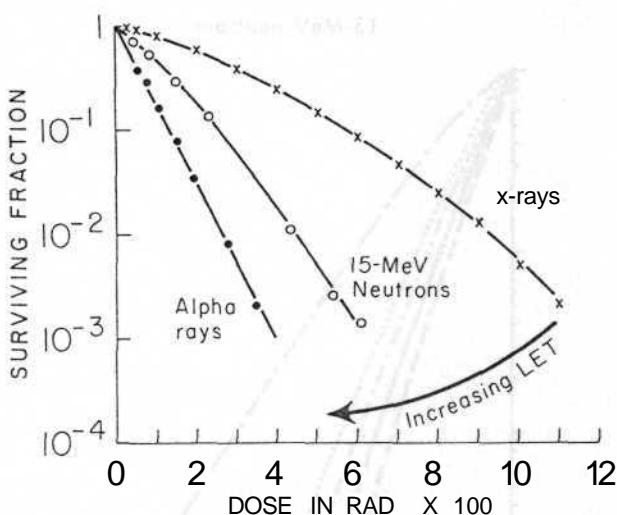


Figure 7.5. Survival curves for cultured cells of human origin exposed to 250-kVp x-rays, 15-MeV neutrons, and 4-MeV  $\alpha$ -particles. As the linear energy transfer of the radiation increases, the slope of the survival curves gets steeper and the size of the initial shoulder gets smaller. (Adapted from Broerse JJ, Barendsen GW, van Kersen GR: Int J Radiat Biol 13:559-572, 1967; and Barendsen GW: Curr Top Radiat Res Q 4: 293-356, 1968, with permission.)

to human, and is the same for mutation as an endpoint as for cell killing.

### THE OPTIMAL LINEAR ENERGY TRANSFER

It is of interest to ask why radiation with an LET of about 100 keV/ $\mu$ m is optimal in terms of producing a biologic effect. At this density of ionization, the average separation between ionizing events just about coincides with the diameter of the DNA double helix (20  $\text{\AA}$  or 2 nm). Radiation with this density of ionization has the highest probability of causing a double-strand break by the passage of a single charged particle, and double-strand breaks are the basis of most biologic effects, as discussed in Chapter 2. This is illustrated in Figure 7.7. In the case of x-rays, which are more sparsely ionizing, the probability of a single track causing a double-strand break is low, and in general more than one track is re-

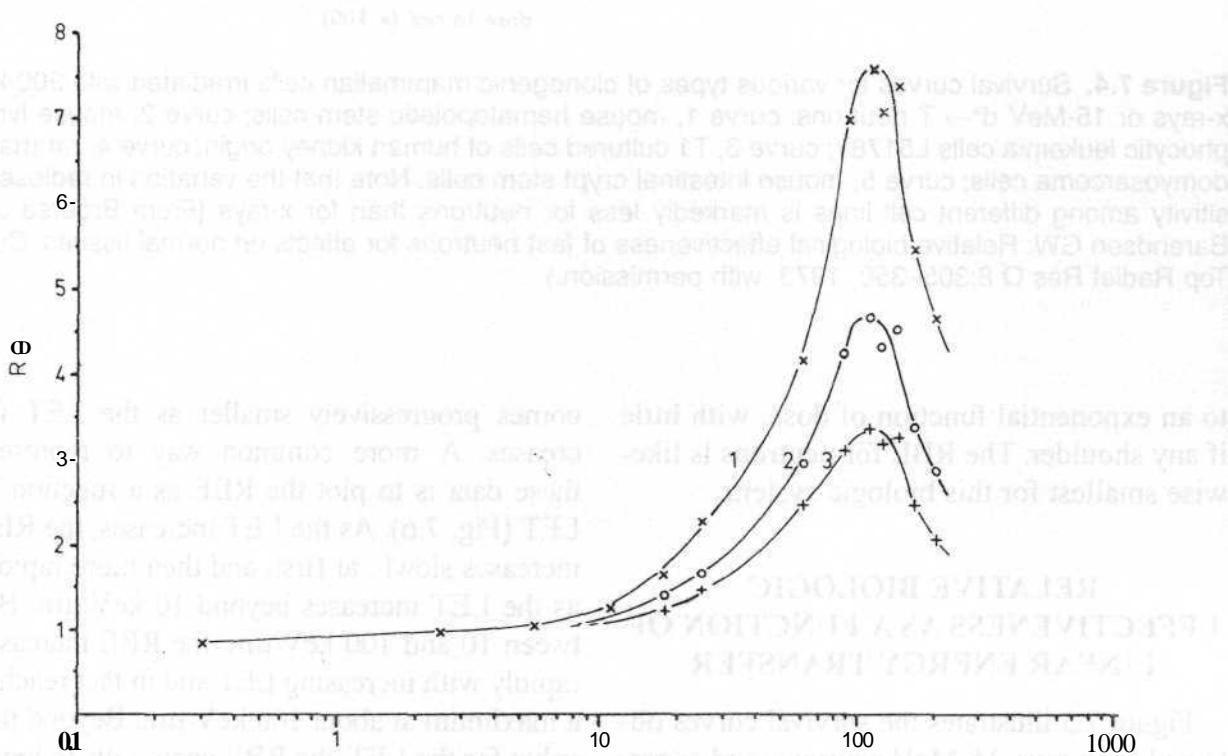
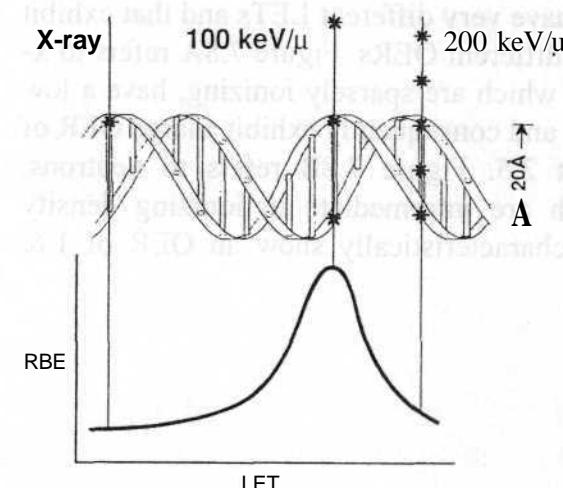


Figure 7.6. Variation of relative biological effectiveness (RBE) with linear energy transfer (LET) for survival of mammalian cells of human origin. The RBE rises to a maximum at an LET of about 100 keV/ $\mu$ m and subsequently falls for higher values of LET. Curves 1, 2, and 3 refer to cell-survival levels of 0.8, 0.1, and 0.01, respectively, illustrating that the absolute value of the RBE is not unique but depends on the level of biologic damage and, therefore, on the dose level. (From Barendsen GW: Curr Top Radiat Res Q 4:293-356, 1968, with permission.)



**Figure 7.7.** Diagram illustrating why radiation with a linear energy transfer of 100 keV/um has the greatest relative biologic effectiveness for cell killing, mutagenesis, or oncogenic transformation. For this transfer, the average separation between ionizing events coincides with the diameter of the DNA double helix (*i.e.*, about 20 Å or 2 nm). Radiation of this quality is most likely to produce a double-strand break from one track for a given absorbed dose.

quired. As a consequence, x-rays have a low biologic effectiveness. At the other extreme, much more densely ionizing radiations (with an LET of 200 keV/u,m, for example) readily produces double-strand breaks, but energy is "wasted" because the ionizing events are too close together. Because RBE is the ratio of doses producing equal biologic effect, this more densely ionizing radiation has a lower RBE than the optimal LET radiation. The more densely ionizing radiation is just as effective *per track*, but less effective per unit dose. It is possible, therefore, to understand why RBE reaches a maximum value in terms of the production of double-strand breaks, because the interaction of two double-strand breaks to form an exchange-type aberration is the basis of most biologic effects. In short, the most biologically effective LET is that at which there is a coincidence between the diameter of the DNA helix and the average separation of ionizing events. Radiations having this optimal LET include neutrons of a few hundred kiloelectron volts, as well as low-energy protons and a-particles.

## FACTORS THAT DETERMINE RELATIVE BIOLOGIC EFFECTIVENESS

The discussion of RBE began with a simple illustration of how this ratio may be determined for neutrons compared with x-rays, using a simple biologic test system with a single, unequivocal endpoint, such as the LD<sub>50</sub> for plant seedlings. Under these circumstances, RBE is conceptually very simple. In the years immediately after World War II it was commonplace to see references to *the RBE* for neutrons, as if it were a single, unique quantity.

Now that more information is available from different biologic systems, many of which allow the researcher to investigate the relationship between biologic response and radiation dose rather than observing one endpoint at a single dose, it is apparent that RBE is a very complex quantity. RBE depends on the following:

- Radiation quality (LET)
- Radiation dose
- Number of dose fractions
- Dose rate
- Biologic system or endpoint

Radiation quality includes the type of radiation and its energy, whether electromagnetic or particulate and whether charged or uncharged.

RBE depends on the dose level and the number of dose fractions (or alternatively, the dose per fraction) because, in general, the shape of the dose-response relationship varies for radiations that differ substantially in their LET.

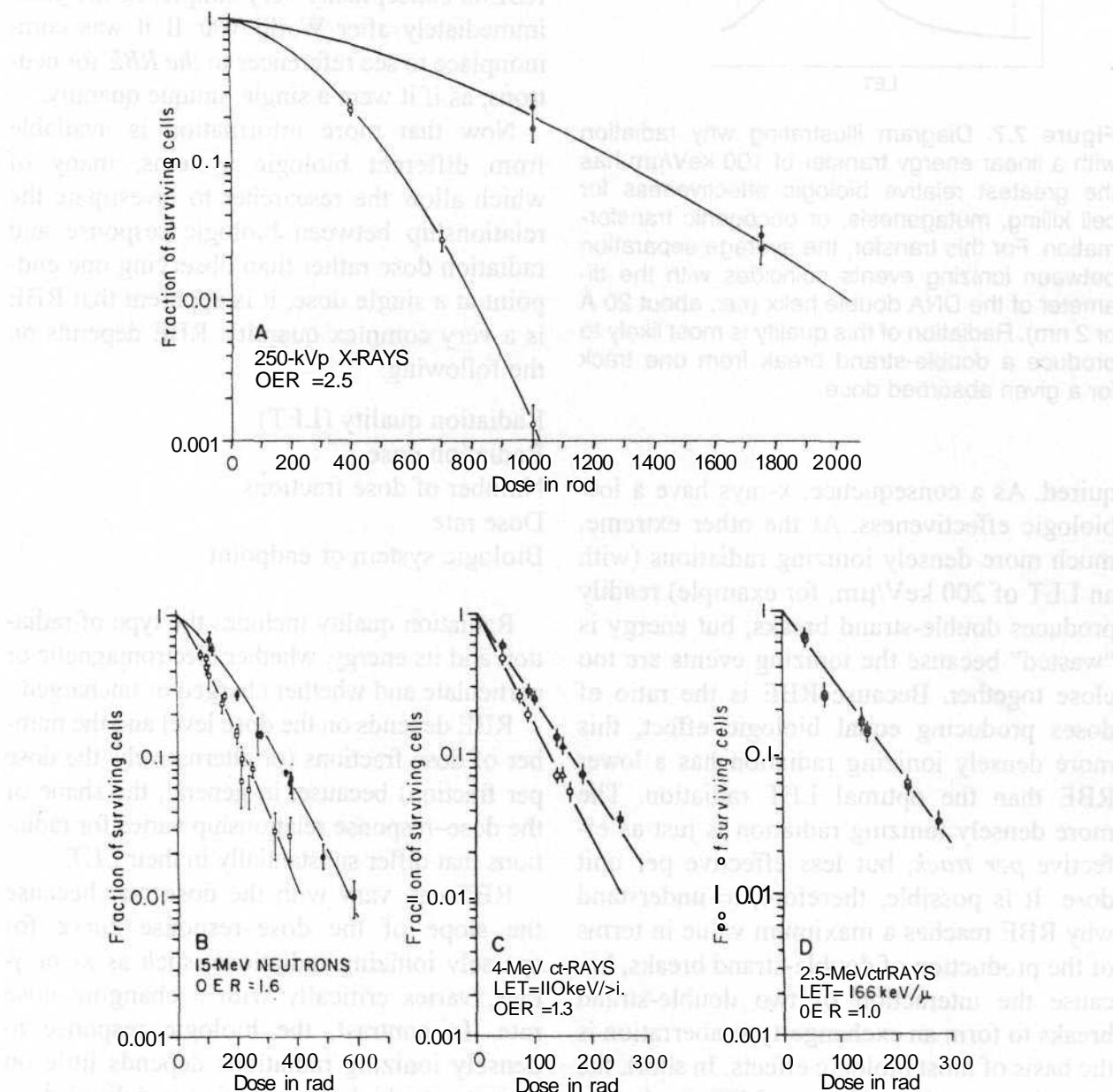
RBE can vary with the dose rate because the slope of the dose-response curve for sparsely ionizing radiations, such as x- or y-rays, varies critically with a changing dose rate. In contrast, the biologic response to densely ionizing radiations depends little on the rate at which the radiation is delivered.

The biologic system or endpoint that is chosen has a marked influence on the RBE values obtained. In general, RBE values are high for tissues that accumulate and repair a great deal of sublethal damage and low for those that do not.

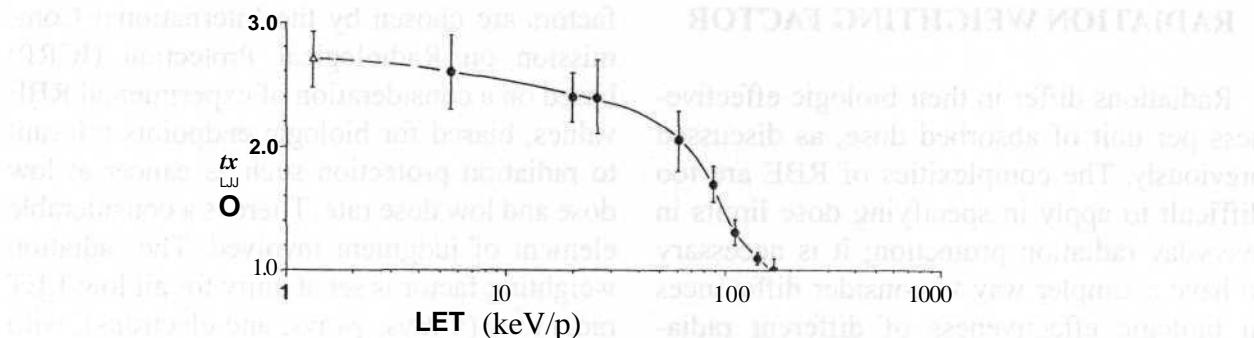
## THE OXYGEN EFFECT AND LINEAR ENERGY TRANSFER

An important relationship exists between LET and the **oxygen enhancement ratio** (OER). Figure 7.8 shows mammalian cell-survival curves for various types of radiation

that have very different LETs and that exhibit very different OERs. Figure 7.8A refers to **X-rays**, which are sparsely ionizing, have a low LET, and consequently exhibit a large OER of about 2.5. Figure 7.8B refers to neutrons, which are intermediate in ionizing density and characteristically show an OER of 1.6.



**Figure 7.8.** Survival curves for cultured cells of human origin determined for four different types of radiation. Open circles refer to aerated and closed circles to hypoxic conditions. A: For 250-kVp x-rays, oxygen enhancement ratio (OER) = 2.5. B: For 15-MeV  $d^+$  ? T neutrons, OER = 1.6. C: For 4-MeV  $\alpha$ -particles, linear energy transfer = 110 keV/jim, OER = 1.3. D: For 2.5-MeV  $\alpha$ -particles, linear energy transfer = 166 keV/ $\mu$ m, OER = 1. (Adapted from Broerse JJ, Barendsen GW: Int J Radiat Biol 13:559, 1967; and Barendsen GW, Koot CJ, van Kersen GR, Bewley DK, Field SB, Parnell CJ: Int J Radiat Biol 10:317-327, 1966, with permission.)



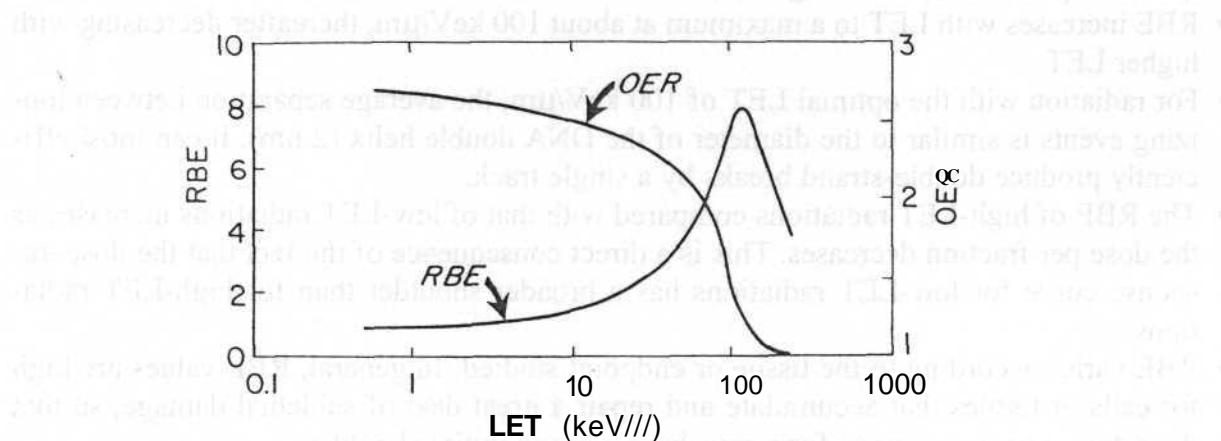
**Figure 7.9.** Oxygen enhancement ratio as a function of linear energy transfer. Measurements were made with cultured cells of human origin. Closed circles refer to monoenergetic charged particles, the open triangle to 250-kVp x-rays with an assumed track average linear energy transfer of 1.3 keV/um. (From Barendsen GW, Koot CJ, van Kersen GR, Bewley DK, Field SB, Parnell CJ: Int J Radiat Biol 10:317-327, 1966, with permission.)

Figure 7.8D refers to 2.5-MeV  $\alpha$ -particles, which have densely ionizing high-LET radiation; survival estimates, whether in the presence or absence of oxygen, fall along a common line, and so the OER is unity. Figure 7.8C contains data for 4-MeV  $\alpha$ -particles, which are slightly less densely ionizing; in this case the OER is about 1.3.

Barendsen and his colleagues have used mammalian cells cultured *in vitro* to investigate the OER for a wide range of radiation types. Their results are summarized in Figure 7.9, in which OER is plotted as a function of LET. At low LET, corresponding to x- or y-

rays, the OER is between 2.5 and 3; as the LET increases, the OER falls slowly at first, until the LET exceeds about 60 keV/jim, after which the OER falls rapidly and reaches unity by the time the LET has reached about 200 keV/jim.

Both OER and RBE are plotted as a function of LET in Figure 7.10. (The curves are taken from the more complete plots in Figures 7.6 and 7.9.) Interestingly, the two curves are virtually mirror images of one another. The optimal RBE and the rapid fall of OER occur at about the same LET value, 100 keV/jim.



**Figure 7.10.** Variation of the oxygen enhancement ratio and the relative biologic effectiveness as a function of the linear energy transfer of the radiation involved. The data were obtained by using T1 kidney cells of human origin, irradiated with various naturally occurring  $\alpha$ -particles or with deuterons accelerated in the Hammersmith cyclotron. Note that the rapid increase of relative biologic effectiveness and the rapid fall of the oxygen enhancement ratio occur at about the same linear energy transfer, 100 keV/um. (Redrawn from Barendsen GW: In: Proceedings of the Conference on Particle Accelerators in Radiation Therapy, pp 120-125. LA-5180-C. US Atomic Energy Commission, Technical Information Center, 1972, with permission.)

## RADIATION WEIGHTING FACTOR

Radiations differ in their biologic effectiveness per unit of absorbed dose, as discussed previously. The complexities of RBE are too difficult to apply in specifying dose limits in everyday radiation protection; it is necessary to have a simpler way to consider differences in biologic effectiveness of different radiations. The term **radiation weighting factor** has been introduced for this purpose. The quantity produced by multiplying the absorbed dose by the weighting factor is called the equivalent dose. When dose is expressed in grays, the equivalent dose is in sieverts (Sv); if dose is in rads, the equivalent dose is in rad equivalent man (rem). Radiation weighting

factors are chosen by the International Commission on Radiological Protection (ICRP) based on a consideration of experimental RBE values, biased for biologic endpoints relevant to radiation protection such as cancer at low dose and low dose rate. There is a considerable element of judgment involved. The radiation weighting factor is set at unity for all low-LET radiations (x-rays, y-rays, and electrons), with a value of 20 for maximally effective neutrons and  $\alpha$ -particles. Detailed values recommended by the International Commission on Radiological Protection are discussed in Chapter 15. Using this system, an absorbed dose of 0.1 Gy (10 rad) of radiation with a radiation weighting factor of 20 would result in an equivalent dose of 2 Sv (200 rem).

## SUMMARY OF PERTINENT CONCLUSIONS

- X- and y-rays are called *sparsely ionizing* because along the tracks of the electrons set in motion, primary ionizing events are well separated in space.
- $\alpha$ -Particles and neutrons are *densely ionizing* because the tracks consist of dense columns of ionization.
- Linear energy transfer (LET) is the energy transferred per unit length of track. Typical values are 0.3 keV/ $\mu$ m for cobalt-60 y-rays, 2 keV/ $\mu$ m for 250-kV x-rays, and 100 to 2,000 keV/ $\mu$ m for heavy charged particles.
- Relative biologic effectiveness (RBE) of some test radiation ( $r$ ) is the ratio  $D_x/D_r$ , in which  $D_x$  and  $D_r$  are the doses of 250-kV x-rays and the test radiation, respectively, required to produce equal biologic effect.
- RBE increases with LET to a maximum at about 100 keV/ $\mu$ m, thereafter decreasing with higher LET.
- For radiation with the optimal LET of 100 keV/ $\mu$ m, the average separation between ionizing events is similar to the diameter of the DNA double helix (2 nm). It can most efficiently produce double-strand breaks by a single track.
- The RBE of high-LET radiations compared with that of low-LET radiations increases as the dose per fraction decreases. This is a direct consequence of the fact that the dose-response curve for low-LET radiations has a broader shoulder than for high-LET radiations.
- RBE varies according to the tissue or endpoint studied. In general, RBE values are high for cells or tissues that accumulate and repair a great deal of sublethal damage, so that their dose-response curves for x-rays have a broad initial shoulder.
- RBE depends on the following:
  - Radiation quality (LET)
  - Radiation dose
  - Number of fractions
  - Dose rate
  - Biologic system or endpoint

- The oxygen enhancement ratio has a value of about 3 for low-LET radiations, falls when the LET rises above about 30 keV/ $\mu$ m, and reaches unity by an LET of about 160 keV/ $\mu$ m.
- The radiation weighting factor depends on LET and is specified by the International Commission on Radiological Protection as a representative RBE at low dose and low dose rate for biologic effects relevant to radiation protection such as cancer induction and heritable effects. It is used in radiologic protection to reduce radiations of different biologic effectiveness to a common scale.
- Equivalent dose is the product of absorbed dose and the radiation weighting factor. If absorbed dose is expressed in grays, equivalent dose is in sieverts. If absorbed dose is in rads, equivalent dose is in rem.

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## Acute Effects of Total-Body Irradiation

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### EARLY LETHAL EFFECTS

### THE PRODROMIC RADIATION SYNDROME

### THE CEREBROVASCULAR SYNDROME

### THE GASTROINTESTINAL SYNDROME

### THE HEMATOPOIETIC SYNDROME

### MEAN LETHAL DOSE AND BONE-MARROW TRANSPLANTS

### TREATMENT OF RADIATION ACCIDENT VICTIMS EXPOSED TO DOSES CLOSE

### TO THE LD<sub>50/60</sub>

### SURVIVORS OF SERIOUS RADIATION ACCIDENTS IN THE UNITED STATES

### RADIATION EMERGENCY ASSISTANCE

### CENTER

### SUMMARY OF PERTINENT CONCLUSIONS

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The effect of ionizing radiation on whole organisms is discussed in this chapter. Data on the acute radiation syndrome have been drawn from many sources. Animal experiments provide the bulk of the data and result in a significant understanding of the mechanisms of death after exposure to total-body irradiation. At the human level, data have been drawn from experiences in radiation therapy and studies of the Japanese survivors of Hiroshima and Nagasaki, the Marshallese accidentally exposed to fallout in 1954, and the victims of the limited number of accidents at nuclear installations, including Chernobyl. From these various sources the pattern of events that follows a total-body exposure to a dose of ionizing radiation has been well documented.

According to the Radiation Accident Registry maintained by the Radiation Emergency Assistance Center at Oak Ridge National Laboratory, there have been 403 radiation accidents worldwide during the period 1944 to 1999. Of these, 19 were criticalities, that is, involving nuclear reactors, but most (303) involved radiation devices, either sealed sources

or x-ray machines, with the remainder (81) involving radioisotopes. These accidents have resulted in 120 deaths; 30 in the United States, 2 in Great Britain, and 32 in the former Union of Soviet Socialist Republics.

### EARLY LETHAL EFFECTS

**Early radiation lethality** generally is considered to be death occurring within a few weeks that can be attributed to a specific high-intensity exposure to radiation. Soon after irradiation, early symptoms appear, which last for a limited period of time; this is referred to as the **prodromal radiation syndrome**. The eventual survival time and mode of death depend on the magnitude of the dose. In most mammals three distinct modes of death can be identified, although in actual accidental exposures some overlap frequently is seen. At very high doses, in excess of about 100 Gy (10,000 rad), death occurs 24 to 48 hours after exposure and appears to result from neurologic and cardiovascular breakdown; this mode of death is known as the **cerebrovascular syndrome**. At intermediate

dose levels, on the order of 5 to 12 Gy (500-1,200 rad), death occurs in a matter of days and is associated with extensive bloody diarrhea and destruction of the gastrointestinal mucosa; this mode of death is known as the **gastrointestinal syndrome**. At low dose levels, on the order of 2.5 to 5 Gy (250-500 rad), death occurs several weeks after exposure and is caused by effects on the blood-forming organs; this mode of death has come to be known as **bone-marrow death**, or the **hematopoietic syndrome**.

The exact cause of death in the cerebrovascular syndrome is by no means clear. In the case of both of the other modes of death—the gastrointestinal and the hematopoietic syndromes—the principal mechanisms that lead to the death of the organism are understood. Death is caused by the depletion of the stem cells of a critical self-renewal tissue: the epithelium of the gut or the circulating blood cells, respectively. The difference in the dose level at which these two forms of death occur and the difference in the time scales involved reflect variations in the population kinetics of the two cell-renewal systems involved and differences in the amount of the damage that can be tolerated in these different systems before death ensues.

### THE PRODROMIC RADIATION SYNDROME

The various symptoms making up the human prodromal syndrome vary with respect to time of onset, maximum severity, and duration, depending on the size of the dose. With doses of a few tens of grays (thousands of

rads), all persons can be expected to show all phases of the syndrome within 5 to 15 minutes of exposure. Reaction might reach a maximum by about 30 minutes and persist for a few days, gradually diminishing in intensity until the prodromal symptoms merge with the universally fatal vascular syndrome or, after a lower dose, with the fatal gastrointestinal syndrome.

At lower doses, dose-response predictions are difficult to make because of the interplay of many different factors. A severe prodromal response usually indicates a poor clinical prognosis and portends at the least a prolonged period of acute hematologic aplasia accompanied by potentially fatal infection, anemia, and hemorrhage.

The signs and symptoms of the human postirradiation syndrome can be divided into two main groups: gastrointestinal and neuromuscular. The gastrointestinal symptoms are anorexia, nausea, vomiting, diarrhea, intestinal cramps, salivation, fluid loss, dehydration, and weight loss. The neuromuscular symptoms include easy fatigability, apathy or listlessness, sweating, fever, headache, and hypotension. At doses that would be fatal to 50% of the population, the principal symptoms of the prodromal reaction are anorexia, nausea, vomiting, and easy fatigability. Immediate diarrhea, fever, and hypotension frequently are associated with supralethal exposure (Table 8.1). One of the Soviet fire-fighters at the Chernobyl reactor accident vividly described the onset of these symptoms as he accumulated a dose of several grays (several hundred rads) working in a high dose-rate area.

TABLE 8.1. *Symptoms of the Prodromal Syndrome*

Neuromuscular	Gastrointestinal
<b>Signs and Symptoms to be Expected at About 50% Lethal Dose</b>	
Easy fatigability	Anorexia Vomiting
<b>Additional Signs to be Expected after Supralethal Doses</b>	
Fever Hypotension	Immediate diarrhea

### THE CEREBROVASCULAR SYNDROME

A total-body dose on the order of 100 Gy (10,000 rad) of y-rays or correspondingly less of neutrons results in death in a matter of hours. At these doses all organ systems also are seriously damaged; the gastrointestinal and the hematopoietic systems both of course are severely damaged and would fail if the person lived long enough, but cerebrovascular damage brings death very quickly, so that the consequences of the failure of the other systems do not have time to be expressed. The symptoms that are observed vary with the species of animal involved and also with level of radiation dose; they are summarized briefly as follows. There is the development of severe nausea and vomiting, usually within a matter of minutes. This is followed by manifestations of disorientation, loss of coordination of muscular movement, respiratory distress, diarrhea, convulsive seizures, coma, and finally death. Only a few instances of accidental human exposure have involved doses high enough to produce a cerebrovascular syndrome; two such cases are described briefly.

In 1964 a 38-year-old man, working in a uranium-235 recovery plant, was involved in an accidental nuclear excursion. He received a total-body dose estimated to be about 88 Gy (8,800 rad) made up of 22 Gy (2,200 rad) of neutrons and 66 Gy (6,600 rad) of y-rays. He recalled seeing a flash and was hurled backward and stunned; he did not lose consciousness, however, and was able to run from the scene of the accident to another building 200 yards away. Almost at once he complained of abdominal cramps and headache, vomited, and was incontinent of bloody diarrheal stools. The next day the patient was comfortable but restless. On the second day his condition deteriorated; he was restless, fatigued, apprehensive, and short of breath and had greatly impaired vision; his blood pressure could only be maintained with great difficulty. Six hours before his death he became disoriented, and his blood pressure could not be maintained; he died 49 hours after the accident.

In a nuclear criticality accident at Los Alamos in 1958, one worker received a total-body dose of mixed neutron and y-radiation estimated to be between 39 and 49 Gy (3,900^4,900 rad). Parts of his body may have received as much as 120 Gy (12,000 rad). This person went into a state of shock immediately and was unconscious within a few minutes. After 8 hours, no lymphocytes were found in the circulating blood, and there was virtually a complete urinary shutdown despite the administration of large amounts of fluids. The patient died 35 hours after the accident.

The exact and immediate cause of death in what is known as the cerebrovascular syndrome is not at all fully understood. Although death usually is attributed to events taking place within the central nervous system, much higher doses are required to produce death if the head alone is irradiated, rather than the entire body; this would suggest that effects on the rest of the body are by no means negligible. It has been suggested that the immediate cause of death is an increase in the fluid content of the brain owing to leakage from small vessels, resulting in a build-up of pressure within the bony confines of the skull.

### THE GASTROINTESTINAL SYNDROME

A total-body exposure of more than 10 Gy (1,000 rad) of y-rays or its equivalent of neutrons commonly leads in most mammals to symptoms characteristic of the gastrointestinal syndrome, culminating in death some days later (usually between 3 and 10 days). The characteristic symptoms are nausea, vomiting, and prolonged diarrhea. Persons lose their appetites and appear sluggish and lethargic. Prolonged diarrhea, extending for several days, usually is regarded as a bad sign because it indicates that the dose received has been more than 10 Gy (1,000 rad), which is inevitably fatal. After a few days, the person shows signs of dehydration, loss of weight, emaciation, and complete exhaustion; death usually occurs in a few days. There is no in-

stance on record of a human having survived a dose in excess of 10 Gy (1,000 rad).

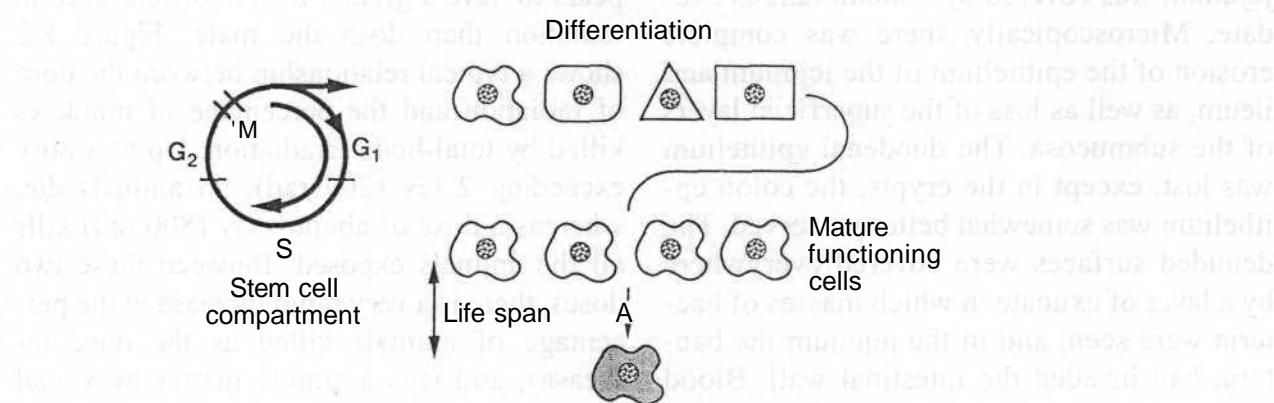
The symptoms that appear and the death that follows are attributable principally to the depopulation of the epithelial lining of the gastrointestinal tract by the radiation. The normal lining of the intestine is a classic example of a self-renewing tissue; Figure 8.1 shows the general characteristics of such a tissue. It is composed of a stem-cell compartment, a differentiating compartment, and mature functioning cells. The structure of the intestinal epithelium is described in some detail in Chapter 18. Dividing cells are confined to the crypts, which provide a continuous supply of new cells; these cells move up the villi, differentiate, and become the functioning cells. The cells at the top of the folds of villi are sloughed off slowly but continuously in the normal course of events, and the villi are continuously replaced by cells that originate from mitoses in the crypts.

A dose of radiation on the order of 10 Gy (1,000 rad) sterilizes a large proportion of the dividing cells in the crypts; a dose of this order of magnitude does not seriously affect the differentiated and functioning cells. As the surface of the villi is sloughed off and rubbed away by normal use, there are no replacement

cells produced in the crypt. Consequently, after a few days, the villi begin to shorten and shrink, and eventually the surface lining of the intestine is completely denuded villi. The rate of cell loss and shrinkage depends on dose. It occurs faster at higher doses than at lower doses. At death the villi are very clearly flat and almost completely free of cells.

The precise time schedule of these events and the time required before the intestine is denuded of cells entirely varies with the species. In small rodents this condition is reached between 3 and 4 days after the dose of radiation is delivered. In large animals, such as the monkey, and probably in humans as well, it does not occur until 5 to 10 days after irradiation. All of the persons who have received a dose large enough for the gastrointestinal syndrome to result in death have already received far more than enough radiation to result in hematopoietic death. Death from a denuding of the gut occurs, however, before the full effect of the radiation on the blood-forming organs has been expressed because of differences in the population kinetics of the stem-cell systems involved.

Before Chernobyl there was probably only one example in the literature of a human suf-



**Figure 8.1.** The classic self-renewal tissue. The stem-cell compartment contains the dividing cells. Of the new cells produced, some maintain the pool and some go on to differentiate and produce mature functioning cells. If the tissue is exposed to radiation, the "Achilles heel" is the stem-cell compartment. Huge doses of radiation are needed to destroy differentiated cells and prevent them from functioning, but modest doses kill some or all of the stem cells, in the sense that they lose their reproductive integrity. Irradiation does not produce an immediate effect on the tissue because it does not affect the functioning cells. The delay between the time of irradiation and the onset of the subsequent radiation syndrome is dictated by the normal life span of the mature functioning cells.

ferring a gastrointestinal death as a result of radiation exposure. In 1946, a 32-year-old man was admitted to the hospital within 1 hour of a radiation accident in which he received a total-body dose of neutrons and  $\gamma$ -rays. The dosimetry is very uncertain in this early accident, and various estimates of total-body exposure range from 11 to 20 Gy (1,100-2,000 rad). In addition, the man's hands received an enormous dose, possibly as much as 300 Gy (30,000 rad). The patient vomited several times within the first few hours of the exposure. On admission, his temperature and pulse rate were slightly elevated; the remainder of the results of his physical examinations were within normal limits. His general condition remained relatively good until the sixth day, on which signs of severe paralytic ileus developed that could be relieved only by continuous gastric suction. On the seventh day, liquid stools that were guaiac-positive for occult blood were noted. The patient developed signs of circulatory collapse and died on the ninth day after irradiation. At the time of death, jaundice and spontaneous hemorrhages were observed for the first time.

At autopsy, the small intestine showed the most striking change. The mucosal surface was edematous and erythematous, and the jejunum was covered by a membranous exudate. Microscopically, there was complete erosion of the epithelium of the jejunum and ileum, as well as loss of the superficial layers of the submucosa. The duodenal epithelium was lost, except in the crypts; the colon epithelium was somewhat better preserved. The denuded surfaces were covered everywhere by a layer of exudate in which masses of bacteria were seen, and in the jejunum the bacteria had invaded the intestinal wall. Blood cultures postmortem yielded *Escherichia coli*.

Several of the firefighters at Chernobyl, including those who received bone-marrow transplants, died between a week and 10 days after exposure, suffering from symptoms characteristic of the gastrointestinal syndrome.

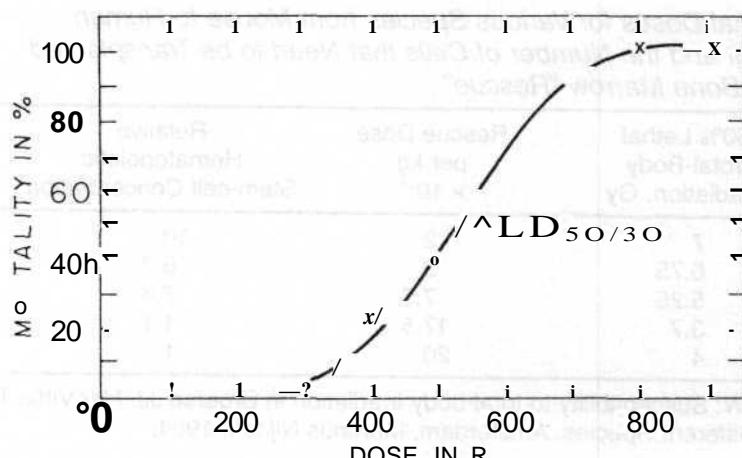
## THE HEMATOPOIETIC SYNDROME

At doses of 3 to 8 Gy (300-800 rad), death, if it occurs, is a result of radiation damage to the hematopoietic system. Mitotically active precursor cells are sterilized by the radiation, and the subsequent supply of mature red blood cells, white blood cells, and platelets is thereby diminished. The time of potential crisis, at which the number of circulating cells in the blood reaches a minimum value, is delayed for some weeks. It is only when the mature circulating cells begin to die off and the supply of new cells from the depleted precursor population is inadequate to replace them that the full effect of the radiation becomes apparent.

The concept of the 50% lethal dose (LD<sub>50</sub>) as an endpoint for scoring radiation death from this cause has been borrowed from the field of pharmacology. The LD<sub>50</sub> is defined as the dose of any agent or material that causes a mortality rate of 50% in an experimental group within a specified period of time.

Within a given population of humans or animals, there are many factors that influence the response of the individual to total-body irradiation. For example, the very young and the old appear to be more radiosensitive than the young adult. The female, in general, appears to have a greater degree of tolerance to radiation than does the male. Figure 8.2 shows a typical relationship between the dose of radiation and the percentage of monkeys killed by total-body irradiation. Up to a dose exceeding 2 Gy (200 rad), no animals die, whereas a dose of about 8 Gy (800 rad) kills all the animals exposed. Between these two doses, there is a very rapid increase in the percentage of animals killed as the dose increases, and it is a simple matter by visual inspection of the graph or by a more sophisticated statistical analysis to arrive at a precise estimate of the LD<sub>50</sub> dose, which in this case is 5.3 Gy (530 rad).

Humans develop signs of hematologic damage and recover from it much more slowly than most other mammals. The peak incidence of human deaths from hematologic



**Figure 8.2.** Mortality rate of rhesus monkeys at 30 days after a single total-body exposure to x-rays. (From Henschke UK, Morton JL: The mortality of rhesus monkeys after single total-body radiation. AJR Am J Roentgenol 77:899-909, 1957, with permission.)

damage occurs at about 30 days after exposure, but deaths continue for up to 60 days. The LD<sub>50</sub> estimates for hematopoietic death for humans are therefore expressed as the LD<sub>50/60</sub>, in contrast to the LD<sub>50/30</sub> for animals, in which peak incidence of death occurs 10 to 15 days after exposure and is complete by 30 days.

A dose of radiation close to the LD<sub>50</sub> results in the prodromal syndrome already described, the chief symptoms of which are nausea and vomiting. A symptom-free interval of time, known as the *latent period*, follows. This is, in fact, a very inappropriate name, because during this period the most important consequences of the radiation exposure, leading to its lethal effects, are in progress. About 3 weeks after the radiation exposure there is onset of chills, fatigue, petechial hemorrhages in the skin, and ulceration of the mouth; epilation also occurs at this time. These symptoms are a manifestation of the depression of blood elements: infections and fever from granulocyte depression and impairment of immune mechanisms, bleeding, and possibly anemia caused by hemorrhage resulting from platelet depression. Anemia from red blood cell depression usually does not occur. Death occurs at this stage unless the bone marrow has begun to regenerate in time. Infection is an important cause of death, but it may be controlled to a large extent by antibiotic therapy.

As a consequence of the reactor accident at Chernobyl, 203 operating personnel, firemen, and emergency workers were hospitalized

suffering from the early radiation syndrome, having received doses in excess of 1 Gy (100 rad). Of these, 35 had severe bone-marrow failure, and 13 of them died. The remainder recovered with conservative medical care.

#### MEAN LETHAL DOSE AND BONE-MARROW TRANSPLANTS

Studies of total-body irradiation have been performed on many species; a few LD<sub>50</sub> values are listed in Table 8.2, ranging from mouse to human. Such studies were popular and important in the 1950s and 1960s, supported largely by the military. In more recent years, total-body irradiation has been of interest from the point of view of bone-marrow transplantation. This interest may stem from the treatment of radiation accidents, such as the Chernobyl disaster, or from the rescue of patients receiving cancer therapy with total-body irradiation, radiolabeled antibodies, or cytotoxic drugs.

Many attempts have been made to estimate the LD<sub>50/60</sub> for humans based on the experiences at Hiroshima and Nagasaki, the total-body irradiation of patients with malignant disease, and the accidents that have occurred at nuclear installations. In a careful summary of all of the available data, Lushbaugh claims that the best estimate is around 3.25 Gy (325 rad) for young healthy adults without medical intervention. There does exist in the literature a surprising number of instances in which young men and women have received total-

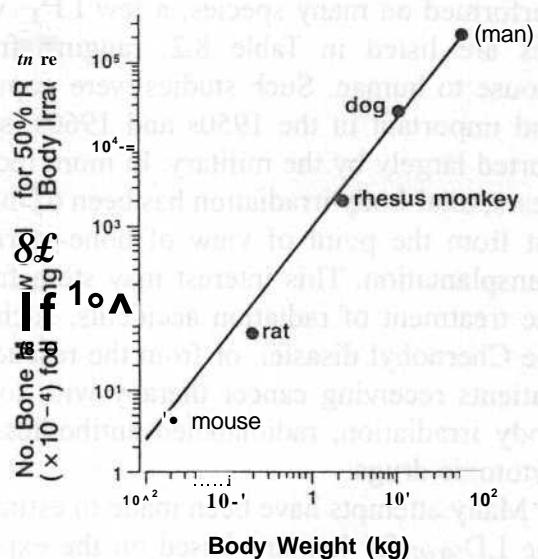
**TABLE 8.2.** *The Fifty Percent Lethal Doses for Various Species from Mouse to Human and the Relation between Body Weight and the Number of Cells that Need to be Transplanted for a Bone Marrow "Rescue"*

Species	Average Body Weight, kg	50% Lethal Total-Body Irradiation, Gy	Rescue Dose per kg $\times 10^{-8}$	Relative Hematopoietic Stem-cell Concentration
Mouse	0.025	7	2	10
Rat	0.2	6.75	3	6.7
Rhesus monkey	2.8	5.25	7.5	7.3
Dog	12	3.7	17.5	1.1
Humans	70	4	20	1

Data from Vriesendorp HM, van Bekkum DW: Susceptibility to total-body irradiation in Broerse JJ, MacVittie T (eds): Response to Total-Body Irradiation in Different Species. Amsterdam, Martinus Nijhoff, 1984.

body irradiation up to a dose of around 4 Gy (400 rad) and recovered under conservative care in a modern well-equipped hospital. The LD<sub>50</sub> for humans quoted in Table 8.2 is the estimate of Vriesendorp and van Bekkum in the Netherlands. In addition to LD<sub>50</sub> data for a number of species, this table also shows estimates of the bone-marrow rescue "dose" re-

quired in a bone-marrow transplant, that is, the number of transplanted bone-marrow cells that are required for a person to recover from a supralethal dose. Larger species are clearly more susceptible to hematopoietic damage than smaller species, as reflected by a lower LD<sub>50</sub>. The bone-marrow transplant experience indicates that this is because of a negative correlation between body weight and hematopoietic stem-cell concentration, that is, the number of hematopoietic stem cells per body unit. The correlation between body weight and the number of bone-marrow cells needed for a rescue is illustrated in Figure 8.3 for a range of species, from mouse to human. Humans require 10 times as many bone-marrow cells per kilogram of body weight as the mouse for a successful bone-marrow rescue after supralethal total-body irradiation, because of the lower concentration of hematopoietic stem cells.



**Figure 8.3.** Correlation between body weight and bone marrow dose for 50% rescue (i.e., number of hematopoietic stem cells required to be transplanted) following supralethal total-body irradiation. (From Vriesendorp HM, van Bekkum DW: Role of total-body irradiation in conditioning for bone marrow transplantation. In Thierfelder S, Rodt H, Kolb HJ (eds): Immunobiology of Bone Marrow Transplantation, pp 349-364. Berlin, Springer Verlag, 1980, with permission.)

#### TREATMENT OF RADIATION ACCIDENT VICTIMS EXPOSED TO DOSES CLOSE TO THE LD<sub>50</sub>/60

If the radiation exposure is known to be less than 4 to 5 Gy (400-500 rad), most experts recommend that the patient be watched carefully but only treated in response to specific symptoms, such as antibiotics for an infection, fresh platelets for local hemorrhage, and so on. Petechial hemorrhages in skin were observed commonly in the Japanese irradi-

ated in 1945 but are not reported so commonly among young white persons exposed accidentally in nuclear power installations. Blood transfusions should not be given prophylactically, because they delay the regeneration of the blood-forming organs.

If the dose is known to have exceeded about 5 Gy (500 rad), then death from the hematopoietic syndrome 3 to 4 weeks later is a real possibility. In some countries isolation and barrier nursing, that is, isolation from others so that they do not come in contact with possible infections while their blood count is low, is recommended. It has been shown in animals that the LD<sub>50</sub> can be raised by a factor of about 2 by the use of antibiotics, and there is no reason to suppose that the same is not true in humans. The important things are to avoid infection, bleeding, and physical trauma during the period in which the circulating blood elements reach a nadir, and to give opportunity for the bone marrow to regenerate.

The area of most discussion and disagreement is the use of bone-marrow transplantation. This technique was used on four Yugoslav scientists who were exposed accidentally in the 1950s to doses initially estimated to be about 7 Gy (700 rad). All of the grafts were rejected, but the exposed persons survived anyway, probably because later estimates indicated that the dose received was much lower, in the region of 4 Gy (400 rad). In fact, many observers claim that the scientists survived in spite of the transplantations, rather than because of them. Figure 8.4 shows the depression and recovery of blood elements in the Yugoslav scientists and also in victims of the famous Y12 reactor accident at Oak Ridge, Tennessee, who received about 4 Gy (400 rad).

In more recent years, bone-marrow transplantation techniques have been greatly improved and, together with growth factors, have been used routinely to "rescue" patients given supralethal doses of radiation for the treatment of leukemia or in preparation for organ transplants. In such cases, of course, the dosimetry is accurate and the doses are just enough to suppress the immunologic response.

Of the Chernobyl accident victims, 13 received bone-marrow transplants (some matched for immune compatibility and some not). In addition, six received fetal liver transplants, but these patients all died early, some of gastrointestinal symptoms. Of the 13 who received bone-marrow transplants, only two survived and one showed autologous bone-marrow repopulation. There was, therefore, only one possible successful transplant that saved a life, and even that result has been questioned.

The situation was made difficult because the doses to which persons had been exposed were not known with any precision. After doses close to the LD<sub>50</sub>, and certainly for higher doses, peripheral lymphocytes disappear before 24 hours, and it is not then possible to estimate total body doses by counting chromosome aberrations in stimulated lymphocytes taken from peripheral blood. Because the U.S. transplant team did not arrive in Chernobyl for some time, biologic dosimetry was never possible for those exposed to higher doses. Consequently, some victims who received bone-marrow transplants already were doomed to die of the gastrointestinal syndrome, having received doses in excess of 10 Gy (1,000 rad).

In fact, the window of dose within which a bone-marrow transplant is useful is very small. Below about 8 Gy (800 rad) an exposed person is likely to survive with careful nursing and an antibiotic screen, because the LD<sub>50</sub> can be approximately doubled by such conservative measures. In such cases, therefore, a transplant is not necessary. Above about 10 Gy (1,000 rad), death from the gastrointestinal syndrome is inevitable, and so a bone-marrow transplant is of no use. This highlights the narrow "window" of dose within which a transplant can be effective (about 8-10 Gy or 800-1000 rad). This is illustrated in Figure 8.5. The urgent need is to develop better methods of *in vivo* biologic dosimetry, because chromosome aberrations in lymphocytes are not always useful in this dose range.

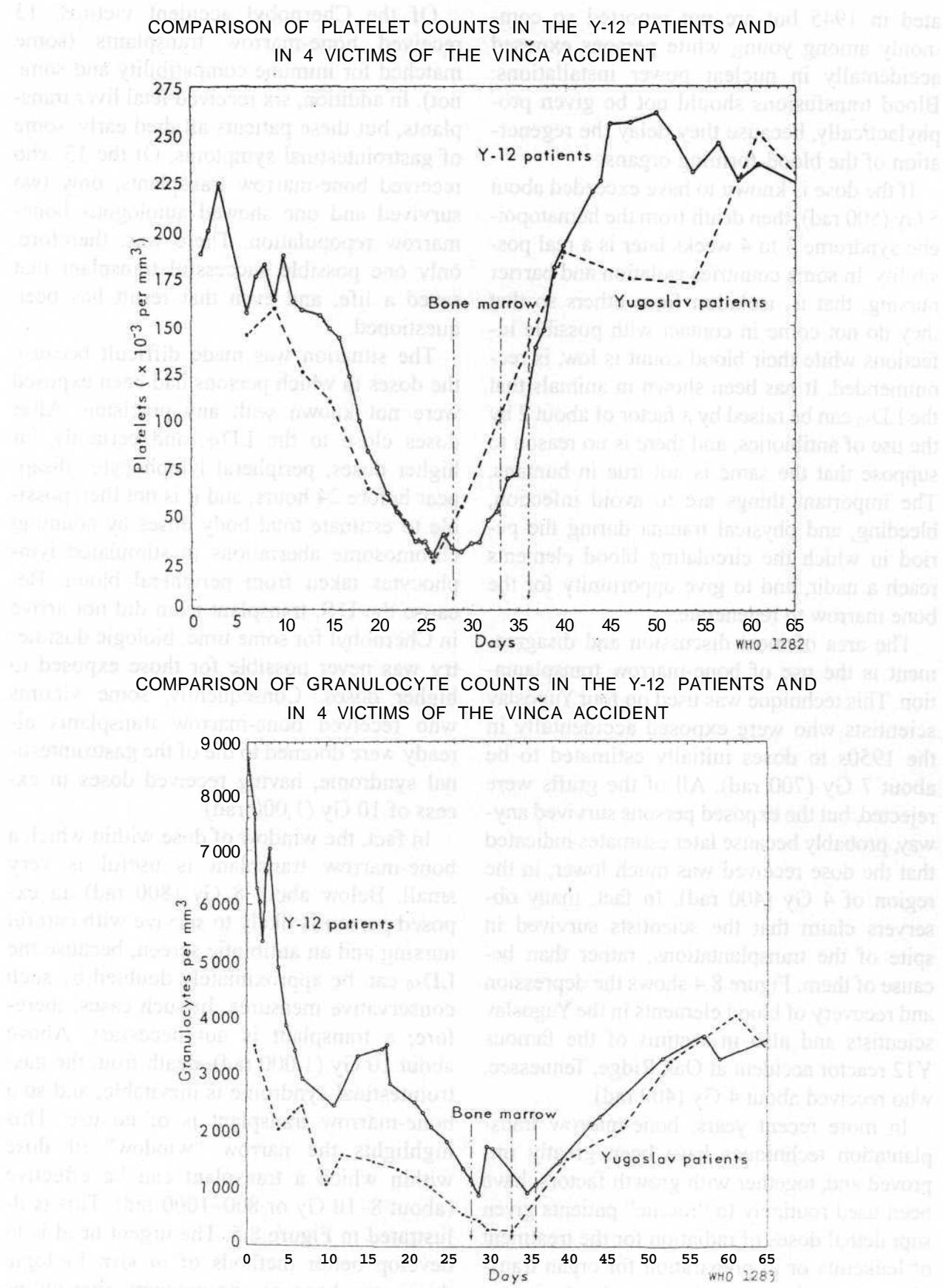
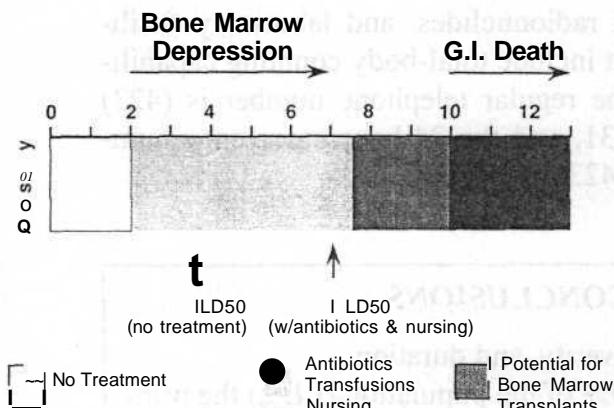


Figure 8.4. Depression and recovery of circulating blood elements in victims of the Y12 reactor accident at Oak Ridge, Tennessee, and four accidentally exposed Yugoslav scientists. (From Andrews GA, Sitterson BW, Kretchmar AL, Brucer M: Diagnosis and Treatment of Acute Radiation Injury, pp 27-48. Geneva, World Health Organization, 1961.)



**Figure 8.5.** Illustrating the narrow window of dose over which bone-marrow transplants might be useful following total-body irradiation. Up to about 8 Gy (800 rad), most persons would survive with antibiotics and careful nursing. Above about 10 Gy (1,000 rad) most persons would die as a consequence of the gastrointestinal syndrome.

## SURVIVORS OF SERIOUS RADIATION ACCIDENTS IN THE UNITED STATES

Over the past 50 years there have been a number of accidents in which small numbers of persons employed in the nuclear program were exposed to total-body or partial-body irradiation. Most occurred in the early days of the nuclear program and involved criticality accidents. The number involved in the United States is about 70 workers in 13 separate accidents.

The long-term survivors have been studied exhaustively over the years. The medical history of these heavily irradiated persons mirrors that of any aging population. The expected high incidences of shortened lifespan, early malignancies after a short latent period, and rapidly progressing lenticular opacities have not been observed. The numbers in any group are small, but the several malignancies, cataracts, and degenerative diseases that have been seen are no more than might be expected in a similar group of unirradiated persons of the same age.

The survivors of the 1958 criticality accident at the Oak Ridge Y12 plant are a case in point. Their blood-cell counts are shown in Figure 8.4. A group of eight workers, ranging

in age from 25 to 56 years, received total-body doses of 0.23 to 3.65 Gy (23-365 rad); five of them received doses above 2 Gy (200 rad). Nevertheless, as of 1999, over 40 years after the accident, none had died of a classic "radiogenic" cancer. There were two cases of lung cancer in very heavy smokers, a meningioma, and prostate cancer in a 70-year-old man. In fact, the only medical finding likely to be radiation-related is bilateral posterior capsular cataracts in two of these patients. Three of the workers who received the biggest doses are retired and in good health.

This highlights the problem of detecting an excess cancer incidence in any small irradiated population. For example, if a group of workers receive a total-body exposure of 3 Gy (300 rad), the biggest dose possible without suffering early death from the hematopoietic syndrome, the excess cancer incidence would be expected to be about 24%. (The cancer risk estimates of the Committee on Biological Effects of Ionizing Radiation and the United Nations Scientific Committee on the Effects of Atomic Radiation based on the Japanese atomic-bomb survivors amount to about 8% per sievert) Thus, the biggest dose to which humans can be exposed and survive doubles the spontaneous cancer incidence. This is difficult to detect in a small group of persons and is likely to be masked by other biologic factors. That is not to say that heavily irradiated persons are not at increased risk, but an excess cancer incidence can be observed only by a careful study of a large population.

## RADIATION EMERGENCY ASSISTANCE CENTER

In the context of radiation accidents, it should be noted that the Medical Sciences Division of the Oak Ridge Institute for Science and Education operates a Radiation Emergency Assistance Center/Training Site (REAC/TS). This is operated on behalf of the U.S. Department of Energy.

REAC/TS provides 24-hour direct or consultative assistance with medical and health

physics problems associated with radiation accidents in local, national, and international incidents. The resources of REAC/TS consist of expertise in cytogenetics for dose assessment, calculation of doses from internally de-

posited radionuclides, and laboratory facilities that include total-body counting capabilities. The regular telephone number is (423) 576-3131, and the 24-hour emergency number is (423) 481-1000.

### SUMMARY OF PERTINENT CONCLUSIONS

- The prodromal syndrome varies in time of onset, severity, and duration.
- At doses close to the dose that would be lethal to 50% of the population (LD50) the principal symptoms of the prodromal syndrome are anorexia, nausea, vomiting, and easy fatigability.
- Immediate diarrhea, fever, or hypotension indicate a supralethal exposure.
- The cerebrovascular syndrome results from a total-body exposure to about 100 Gy (10,000 rad) of  $\gamma$ -rays and results in death in 30 to 50 hours. The pause of death may be changes in permeability of small blood vessels in the brain.
- The gastrointestinal syndrome results from a total-body exposure to about 10 Gy (1,000 rad). Death occurs in about 9 days in humans because of depopulation of the epithelial lining of the gastrointestinal tract.
- The hematopoietic syndrome results from total-body exposure of 3 to 8 Gy (300-800 rad). The radiation sterilizes some or all of the mitotically active precursor cells. Symptoms result from lack of circulating blood elements 3 weeks or more later.
- The LD50 for humans is 3 to 4 Gy (300-400 rad) for young adults without medical intervention. It may be less for the young or the old.
- Some persons who would otherwise die may be saved by antibiotics, platelet infusions, or bone-marrow transplants.
- In animals, the LD50 can be raised by a factor of 2 by appropriate treatment, including careful nursing and antibiotics.
- The dose window over which bone-marrow transplants may be useful is narrow, namely, 8 to 10 Gy (800-1,000 rad).
- Heavily irradiated survivors of accidents in the nuclear industry have been followed for many years; their medical history mirrors that of any aging population. A high incidence of shortened lifespan, early malignancies after a short latency, and rapidly progressing cataracts have not been observed.

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## Radioprotectors

THE DISCOVERY OF RADIOPROTECTORS  
MECHANISM OF ACTION  
DEVELOPMENT OF MORE EFFECTIVE  
COMPOUNDS  
AMIFOSTINE (WR-2721) AS A  
RADIOPROTECTOR IN RADIOTHERAPY

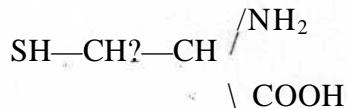
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RADIOPROTECTORS AND  
CHEMOTHERAPY  
SUMMARY OF PERTINENT  
CONCLUSIONS

### THE DISCOVERY OF RADIOPROTECTORS

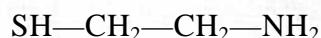
Some substances, although they do not directly affect the radiosensitivity of cells, nevertheless may protect whole animals because they cause vasoconstriction or in some way upset normal processes of metabolism to such an extent that the oxygen concentration in critical organs is reduced. Because cells are less sensitive to x-rays under hypoxia, this confers a measure of protection. Examples are sodium cyanide, carbon monoxide, epinephrine, histamine, and serotonin. Such compounds are not really radioprotectors *per se* and are not discussed further here.

The most remarkable group of true radioprotectors are the sulphydryl compounds. The simplest is **cysteine**, a sulphydryl compound containing a natural amino acid, the structure of which is



In 1948, Part discovered that cysteine could protect mice from the effects of total-body x-radiation if the drug was injected or ingested in large amounts before the radiation expo-

sure. At about the same time, Bacq and his colleagues in Europe independently discovered that cysteamine could also protect animals from total-body irradiation. This compound has a structure represented by



Animals injected with cysteamine to concentrations of about 150 mg/kg require doses of x-rays 1.8 times larger than control animals to produce the same mortality rate. This factor of 1.8 is called the *dose-reduction factor*, defined as

$$U_{\text{D}\nu r} = \frac{\text{Dose of radiation in the presence of the drug}}{\text{Dose of radiation in the absence of the drug}}$$

to produce a given level of lethality.

### MECHANISM OF ACTION

Many similar compounds have been tested and found to be effective as radioprotectors. The most efficient tend to have certain structural features in common: a free SH group (or potential SH group) at one end of the molecule and a strong basic function such as amine

or guanidine at the other end, separated by a straight chain of two or three carbon atoms. Sulphydryl compounds are efficient radioprotectors against sparsely ionizing radiations, such as x- or y-rays.

The mechanisms most implicated in SH-mediated cytoprotection include:

1. Free-radical scavenging that protects against oxygen-based free-radical generation by ionizing radiations or chemotherapy agents such as alkylating agents.
2. Hydrogen-atom donation to facilitate direct chemical repair at sites of DNA damage.

Chapter 1 includes a discussion of the chain of events between the absorption of a photon and the eventual biologic damage, which includes the production of free radicals, which are highly reactive species. If

these free radicals are scavenged before they can interact with biologic molecules, the effect of the radiation is reduced. This process is illustrated in Figure 9.1.

The protective effect of sulphydryl compounds tends to parallel the oxygen effect, being maximal for sparsely ionizing radiations (*e.g.*, x- or y-rays) and minimal for densely ionizing radiations (*e.g.*, low-energy  $\alpha$ -particles). It might be predicted that with effective scavenging of all free radicals the largest possible value of dose-reduction factor would equal the oxygen enhancement ratio, with a value of 2.5 to 3.0.

This simple description of the mechanism of action of sulphydryl radioprotectors is intellectually satisfying, but it is clearly not the whole story, because radioprotectors of this class have more effect with densely ionizing radiations (such as neutrons) than would be expected. Other factors must be involved that are not fully understood.

## DEVELOPMENT OF MORE EFFECTIVE COMPOUNDS

It is not surprising that the discovery in 1948 of a compound that offered protection against radiation excited the interest of the U.S. Army, because the memory of Nagasaki and Hiroshima was vivid in the years immediately after World War II. Although cysteine is a radioprotector, it is also toxic and induces nausea and vomiting at the dose levels required for radioprotection. A development program was initiated in 1959 by the United States Army in studies conducted at the Walter Reed Institute of Research to identify and synthesize drugs capable of conferring protection to individuals in a radiation environment, but without the debilitating toxicity of cysteine or cysteamine. Over 4,000 compounds were synthesized and tested. At an early stage the important discovery was made that the toxicity of the compound could be greatly reduced if the **sulphydryl** group was covered by a phosphate group. This is illustrated in Table 9.1. The 50% lethal dose of the

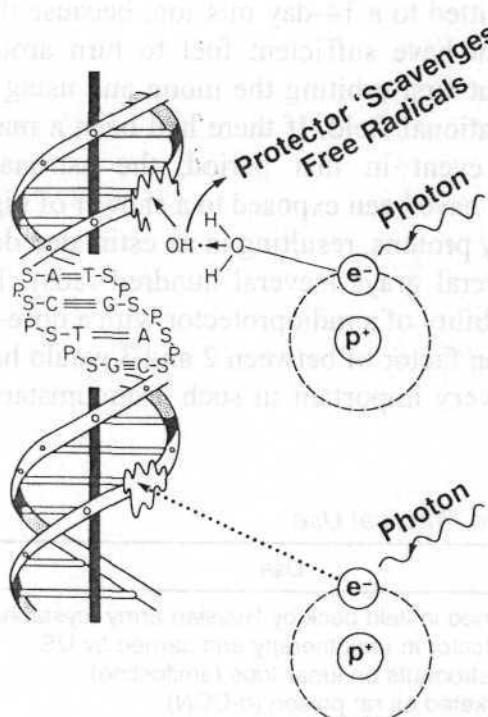


Figure 9.1. Radioprotectors containing a sulphydryl group exert their effect by scavenging free radicals and by reducing free-radical damage to DNA. They are most effective for low linear energy transfer radiations, becoming progressively less effective with increasing linear energy transfer because the amount of local damage is so great.

**TABLE 9.1.** Effect of adding a Phosphate-Covering Function on the Free Sulphydryl of 3-Mercaptoethylamine (MEA)

Drug	Formula	Mean 50% Lethal Dose (Range) in Mice	Dose-reduction Factor
MEA	NH <sub>2</sub> -CH-CH <sub>2</sub> -SH	343 (323-364)	1.6 at 200 mg/kg
MEA-PO <sub>3</sub>	NH <sub>2</sub> -CH <sub>2</sub> -CH-SH <sub>2</sub> PO <sub>3</sub>	777 (700-864)	2.1 at 500 mg/kg

compound in animals can be doubled and the protective effect in terms of the dose-reduction factor greatly enhanced if the SH group in cysteamine is covered by a phosphate. This tends to reduce systemic toxicity. Once in the cell, the phosphate group is stripped, and the SH group begins scavenging for free radicals.

The structures of three typical compounds of the more than 4,000 synthesized in the Walter Reed series are shown in Table 9.2. The first compound, WR-638, called *cystaphos*, was said to be carried routinely in the field pack of Soviet infantry in Europe during the era of the Cold War for use in the event of a nuclear conflict. Its usefulness must be largely psychological, because the compound was carried as a tablet to be administered orally, although in fact these sulphydryl compounds break down in stomach acid and are effective only if administered intravenously or intraperitoneally. A further factor, of course, is that such compounds protect only from sparsely ionizing radiation; consequently, they would offer little protection against the prompt release of neutrons produced by the detonation of a nuclear device. They would be

effective only against the  $\gamma$ -rays from the resulting fallout.

The second compound, WR-2721, now known as *amifostine*, is perhaps the most effective of those synthesized in the Walter Reed series. It gives good protection to the blood-forming organs, as can be seen by the dose-reduction factor for 30-day death in mice, which approaches the theoretic maximum value of 3. It was probably the compound carried by U.S. astronauts on their trips to the moon, to be used if a solar event occurred. On these missions, if the space vehicle left earth's orbit and began coasting toward the moon, the astronauts were committed to a 14-day mission, because they did not have sufficient fuel to turn around without first orbiting the moon and using its gravitational field. If there had been a major solar event in that period, the astronauts would have been exposed to a shower of high-energy protons, resulting in an estimated dose of several grays (several hundred rads). The availability of a radioprotector with a dose-reduction factor of between 2 and 3 would have been very important in such a circumstance.

**TABLE 9.2.** Three Protectors in Practical Use

Compound	Structure	Use
WR-638	NH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> SPO <sub>3</sub> HNa	Carried in field pack by Russian army ( <i>cystaphos</i> )
WR-2721	NH <sub>2</sub> (CH <sub>2</sub> ) <sub>3</sub> NHCH <sub>2</sub> CH <sub>2</sub> SPO <sub>3</sub> H <sub>2</sub>	Protector in radiotherapy and carried by US astronauts on lunar trips ( <i>amifostine</i> )
WR-1607	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>9</sub> NHCH <sub>2</sub> CH <sub>2</sub> SSO <sub>3</sub> H	Marketed as rat poison (d-CON)

*Comparison of Hematopoietic and Gastrointestinal Dose Reduction Factors in Mice for the Three Compounds Listed Above*

Compound	Drug Dose, mg/kg	Dose-reduction Factor	
		7 Days	30 Days
WR-638	500	1.6	2.1
WR-2721	900	1.8	2.7
WR-1607	10	—	2.1

As it turned out, no major solar event occurred during any manned lunar mission. Amifostine also has a potential in radiotherapy, which is discussed in more detail subsequently.

The third compound, WR-1607, has a structure similar to the other two but is in fact marketed as the rat poison d-CON. It kills by producing cardiac arrest. It is a much more effective radioprotector than either of the others listed in Table 9.2, producing equivalent protection at one hundredth of the dose, but it is not usable because of its toxicity. This compound is included in Table 9.2 because it illustrates how a small change in structure can result in a dramatic change of properties, and because it also points to the potential dose-limiting cytotoxicity of this series of sulfhydryl compounds. For instance, the dose-limiting toxicity of amifostine is hypotension.

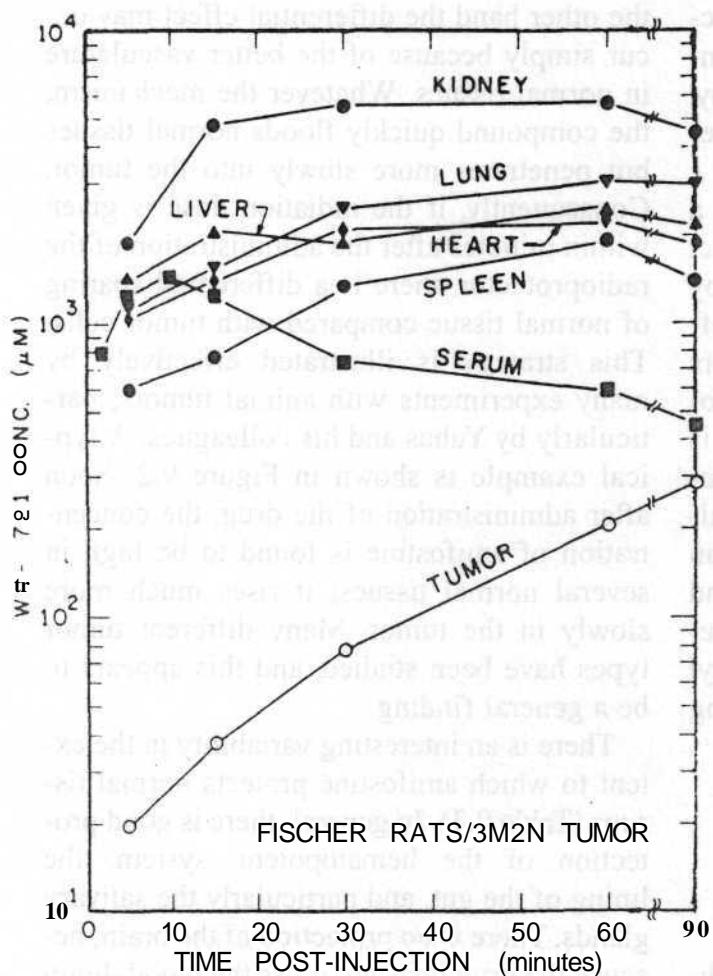
### AMIFOSTINE AS A RADIOPROTECTOR IN RADIOTHERAPY

Amifostine (WR-2721) is a phosphorothioate that is nonreactive and does not readily permeate cells, primarily because of its terminal phosphorothioic acid group. It is therefore a prodrug. If dephosphorylated by the enzyme alkaline phosphatase, which is present in high concentrations in normal tissues and capillaries, it is converted to the active metabolite designated WR-1065. This metabolite readily enters normal cells by facilitated diffusion and scavenges free radicals generated by ionizing radiations or by some chemotherapy agents such as alkylating agents. Radioprotectors have a number of potential applications in radiotherapy. For use in total-body irradiation the drug is administered at maximum tolerable concentration immediately before the radiation dose is delivered. Protection of normal tissues versus tumors is achieved through a differential uptake and conversion of amifostine to WR-1065 in tumors. There is evidence of active transport of the drug into normal tissues as described previously, with only passive diffusion into tumors; on

the other hand the differential effect may occur simply because of the better vasculature in normal tissues. Whatever the mechanism, the compound quickly floods normal tissues but penetrates more slowly into the tumor. Consequently, if the radiation dose is given within minutes after the administration of the radioprotector, there is a differential sparing of normal tissue compared with tumor cells. This strategy is illustrated effectively by many experiments with animal tumors, particularly by Yuhas and his colleagues. A typical example is shown in Figure 9.2. Soon after administration of the drug, the concentration of amifostine is found to be high in several normal tissues; it rises much more slowly in the tumor. Many different tumor types have been studied, and this appears to be a general finding.

There is an interesting variability in the extent to which amifostine protects normal tissues (Table 9.3). In general, there is good protection of the hematopoietic system, the lining of the gut, and particularly the salivary glands. There is no protection of the brain, because the drug does not cross the blood-brain barrier, and a disappointing level of protection is seen in the lung. The mechanism by which amifostine exerts a differential effect between normal tissues and tumors is not entirely clear. Part of the reason for the slower uptake in tumors may be that they generally have poorly developed vascular systems, but this is clearly not the whole story. An essential feature of the design of successful radioprotectors is that they must be hydrophilic (*i.e.*, more soluble in water than in lipids). Lipophilic radioprotectors do not show this differential uptake between normal tissues and tumors. Therefore it may be that the difference in uptake of amifostine between normal tissues and tumors is caused in some way by a difference in the membrane structure of the tumor cells, which allows hydrophilic drugs to penetrate only slowly.

Figure 9.3 shows an autoradiograph of an animal given a radioactive-labeled amifostine 20 minutes before sacrifice. A number of features already discussed are illustrated dramat-



**Figure 9.2.** Serum, tissue, and tumor concentration of the radioprotector amifostine (WR-2721) as a function of time after intraperitoneal administration of the drug (200 mg/kg). The radioprotector penetrates more slowly into the tumor than into many normal tissues, so that if the radiation dose is delivered soon after the administration of the drug, there is a differential protection of normal tissues. Similar results have been shown in a wide variety of transplantable tumors in laboratory animals. [From Yuhas J: Active versus passive absorption kinetics as the basis for selective protection of normal tissues by S-2-(3-aminopropylamino)-ethyl-phosphorothioic acid. *Cancer Res* 40:1519-1524, 1980, with permission.]

**TABLE 9.3.** Summary of Normal-Tissue Responsiveness to Protection by WR-2721

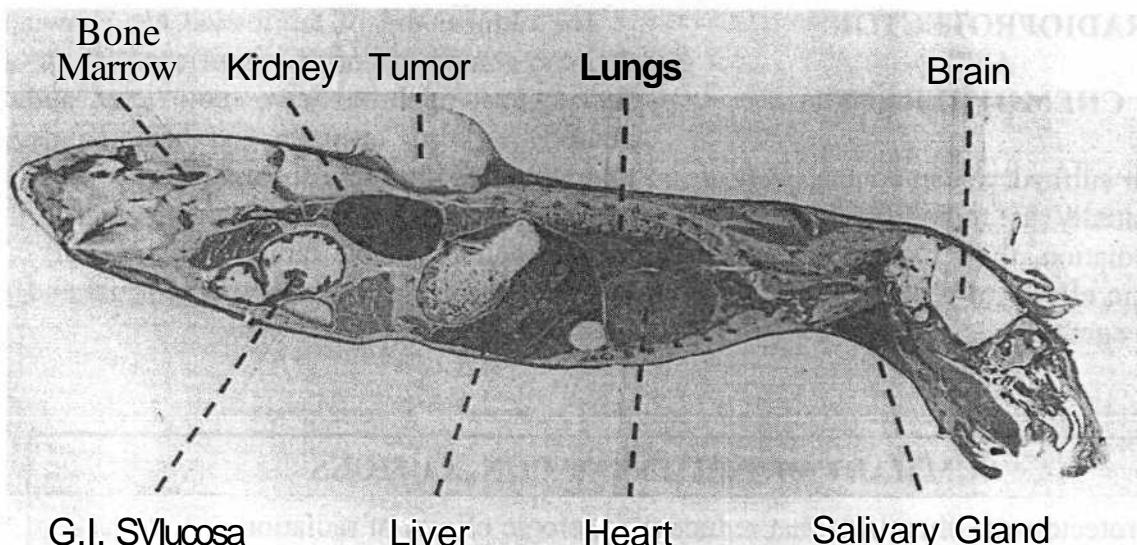
Tissues Protected <sup>a</sup>	Tissues not Protected
Bone marrow (2.4-3)	Brain
Immune system (1.8-3.4)	Spinal cord
Skin (2-2.4)	
Small intestine (1.8-2)	
Colon (1.8)	
Lung (1.2-1.8)	
Esophagus (1.4)	
Kidney (1.5)	
Liver (2.7)	
Salivary gland (2G)	
Oral mucosa (>1)	
Testes (2.1)	

<sup>a</sup>Numbers in parentheses are the dose reduction factors or factor increases in resistance associated with WR-2721 injection.

From Yuhas JM, Spellman JM, Culo F: The role of WR2721 in radiotherapy and/or chemotherapy. In Brady L (ed): *Radiation Sensitizers*, pp 303-308. New York, Masson, 1980, with permission.

ically in this section. First, the tumor (a transplanted EMT6 tumor) is entirely cold—it has not taken up the drug at all. The bone marrow, gut, and salivary glands are very black, indicating a high uptake of the drug. The lungs show an intermediate uptake.

The clinical exploitation of radioprotectors has been slow in coming. Phase I toxicity trials of amifostine conducted in humans in the United States have shown that the dose-limiting toxicity is hypotension. Other symptoms include sneezing and somnolence. These undesirable side effects tend to limit the amount of drug given to levels lower than necessary to achieve maximum protection, based on animal experiments. A randomized clinical trial of a radioprotector has been conducted in mainland China. One hundred patients with inoperable unresectable or recurrent adenocarcinoma of the rectum were stratified and randomized to amifostine plus radiotherapy



**Figure 9.3.** Autoradiograph of a mouse showing the distribution of amifostine, labeled with radioactive sulfur-35 at 6 minutes after intravenous injection. The greatest densities are seen in the kidney, liver, intestinal mucosa, and submandibular salivary gland. The brain shows little drug concentration because amifostine is hydrophilic and does not cross the blood-brain barrier. The drug has also not concentrated in the tumor (a transplanted EMT6). (From Utley JF, Marlowe C, Waddell WJ: Distribution of 35S-labeled WR-2721 in normal and malignant tissues of the mouse. Radiat Res 68:284-291, 1976, with permission.)

or to radiotherapy alone. The radioprotector was administered 15 minutes before radiotherapy, 4 days a week for 5 weeks. Those patients pretreated with amifostine showed protection of the skin, mucous membrane, bladder, and pelvic structures against late moderate and severe reactions. In the radiation-only group, 5 of 37 patients exhibited such reactions; with none receiving the radioprotector the difference is statistically significant. At the same time there was no apparent protection afforded to the tumor.

One of the difficult and worrisome factors in the experimental use of radioprotectors in the clinic is that their use is not fail-safe. To exploit a benefit, radiation doses must be *increased* with the confidence that the normal tissues are protected and that the extra dose can improve tumor response. If radioprotection does not occur, unacceptable normal tissue injury results. This requires courage on the part of the investigator.

A more modest but achievable goal is to use a radioprotector to reduce the troublesome side effects of radiation therapy. The RTOG (Radiotherapy Oncology Group) has conducted a

phase III randomized clinical trial, which demonstrated the efficacy of amifostine in reducing xerostomia in head-and-neck cancer patients receiving radiotherapy without prejudice to early tumor control. The drug was administered daily, 30 minutes before each dose fraction in a multifraction regimen. Three months posttreatment, the incidence of xerostomia was significantly reduced in those patients treated with amifostine. There was an improvement in the patients' assessments of such symptoms as dry mouth and difficulty in eating or speaking and in the need for fluids and oral comfort aids. There was no difference in locoregional tumor control between patients who received the radioprotector and those who did not. Giving the amifostine only 30 minutes before each treatment was designed to exploit the slower rate at which the drug penetrates tumors relative to normal tissues.

Although the emphasis in the development of amifostine was to protect against cell killing, this compound also protects against mutagenesis in cell culture and animal systems, and against radiation-induced carcinogenesis in mouse models.

## RADIOPROTECTORS AND CHEMOTHERAPY

Although sulfhydryl compounds were developed initially as radioprotectors against ionizing radiation, they also protect against the cytotoxic effects of a number of chemotherapeutic agents.

The clinical use of amifostine has shown that the compound offers significant protection against nephrotoxicity, ototoxicity, and neuropathy from cisplatin and hematologic toxicity from cyclophosphamide. The same experimental studies indicated no obvious antitumor activity of the radioprotector, implying a differential uptake between normal and malignant tissues.

### SUMMARY OF PERTINENT CONCLUSIONS

- Radioprotectors are chemicals that reduce the biologic effects of radiation.
- The sulfhydryl compounds cysteine and cysteamine were discovered early but are toxic. If the SH group is covered by a phosphate group, toxicity is reduced.
- The dose-reduction factor is the ratio of radiation doses required to produce the same biologic effect in the absence and presence of the radioprotector.
- The best available radioprotectors can attain dose-reduction factor values of 2.0 to 2.7 for bone-marrow death in mice irradiated with x-rays.
- The mechanism of action is the scavenging of free radicals and restitution of free-radical damage.
- Dose-reduction factor values close to the oxygen enhancement ratio are possible for x-rays, but the effectiveness of radioprotectors decreases with increasing linear energy transfer.
- Radioprotectors were carried to the moon by U.S. astronauts to be used in the event of a solar flare. During the era of the Cold War, it is said that Soviet infantry in Europe carried radioprotectors for use in a possible nuclear war.
- More than 4,000 compounds have been synthesized by the U.S. Army in studies conducted at the Walter Reed Institute of Research. Amifostine (WR-2721) appears to be the best for radiotherapy.
- Amifostine (WR-2721) is a prodrug that is unreactive and penetrates poorly into cells until it is dephosphorylated by the enzyme alkaline phosphatase to the active metabolite WR-1065.
- Bone marrow, the gut, and the salivary glands are well protected by amifostine; the lung is protected poorly and the brain not at all because the drug is hydrophilic and does not cross the blood-brain barrier.
- Amifostine is used in radiotherapy because it floods many normal tissues rapidly after administration but penetrates tumors much more slowly. The strategy is to begin irradiation soon after administration of the drug to exploit a differential effect.
- A clinical trial of amifostine in mainland China has shown protection of normal tissues, with no evidence of protection of the tumor in patients with adenocarcinoma of the rectum treated with radiation. The drug was administered 15 min before radiotherapy.
- An RTOG phase III trial has demonstrated the efficacy of amifostine in reducing xerostomia in patients with head-and-neck cancer receiving radiation therapy without affecting locoregional control. The radioprotector was administered 30 min before radiation.
- Amifostine is useful as a protector for chemotherapy as well as radiotherapy. It is reported to offer protection against nephrotoxicity, ototoxicity, and neuropathy from cisplatin and hematologic toxicity from cyclophosphamide, with reduction of tumor **activity**.

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# 10

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## Radiation Carcinogenesis

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### DETERMINISTIC AND STOCHASTIC

#### EFFECTS

### CARCINOGENESIS: THE HUMAN EXPERIENCE

### CARCINOGENESIS IN LABORATORY ANIMALS

### THE LATENT PERIOD

### ASSESSING THE RISK

### COMMITTEES CONCERNED WITH RISK

#### ESTIMATES AND RADIATION PROTECTION

### LEUKEMIA

### THYROID CANCER

### BREAST CANCER

### LUNG CANCER

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### BONE CANCER

### SKIN CANCER

### QUANTITATIVE RISK ESTIMATES FOR RADIATION-INDUCED CANCER DOSE AND DOSE-RATE EFFECTIVENESS FACTOR

### SUMMARY OF RISK ESTIMATES

### SECOND MALIGNANCIES IN RADIOTHERAPY PATIENTS CANCER RISKS IN NUCLEAR-INDUSTRY WORKERS CHILDHOOD CANCER AFTER RADIATION EXPOSURE *IN UTERO*

### SUMMARY OF PERTINENT CONCLUSIONS

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## DETERMINISTIC AND STOCHASTIC EFFECTS

If cellular damage occurs as a result of radiation that is not adequately repaired, it may prevent the cell from surviving or reproducing or result in a viable cell that has been modified, that is, suffered a change or mutation that it retains as a legacy of the radiation exposure.

The two outcomes have profoundly different implications for the person of whom the cell is a part.

Most organs or tissues of the body are unaffected by the loss of a few cells; but if the number lost is sufficiently large, there is observable harm, reflecting the loss of tissue function. The probability of causing such harm is zero at small radiation doses, but above some level of dose, called the *threshold*

*dose*, the probability increases rapidly with dose to 100%. Above the threshold the severity of harm also increases with dose. Effects such as this, previously called *nonstochastic*, now are called *deterministic*. A deterministic effect has a threshold of dose, and the severity of the effect is dose-related. Radiation-induced cataracts are an example.

The outcome is very different if the irradiated cell is viable but modified. Carcinogenesis and hereditary effects fall into this category. If somatic cells are exposed to radiation, the probability of cancer increases with dose, probably with no threshold. But the severity of the cancer is not dose-related. A cancer induced by 1 Gy (100 rad) is no worse than one induced by 0.1 Gy (10 rad), but of course the probability of its induction is increased. This category of effect is called *stochastic*, a word that has been given a special meaning in radi-

ation protection but, in general, just means "random." If the radiation damage occurs in germ cells, mutations may occur that could cause deleterious effects in future generations. Again, there is probably no threshold, and the severity of hereditary effect is not dose-related, although the probability of it occurring is.

The belief that stochastic effects have no dose threshold is based on the molecular mechanisms involved. There is no reason to believe that even a single x-ray photon could not result in a base change leading to a mutation that could cause cancer or a hereditary defect. For this reason it is considered prudent to assume that no dose is too small to be effective.

The two types of effects are summarized as follows:

Deterministic effect: severity increases with dose; practical threshold; *e.g.*, cataract

Stochastic effect: severity independent of dose; probability of it occurring increases with dose; no threshold; *e.g.*, cancer

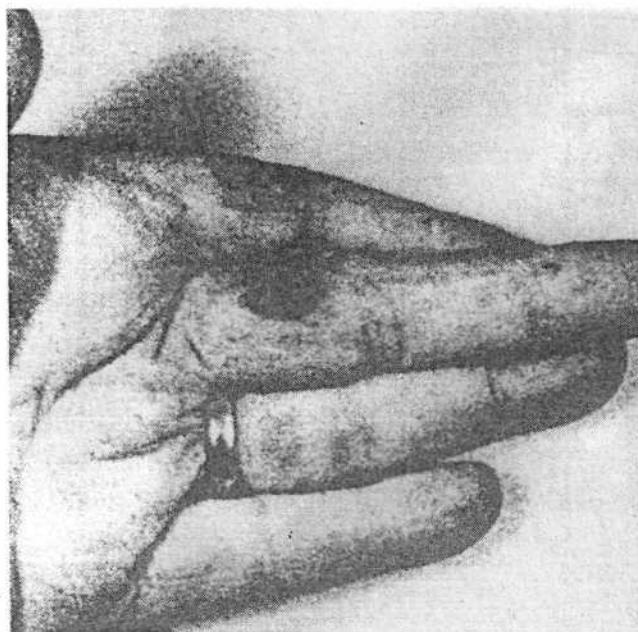
### CARCINOGENESIS: THE HUMAN EXPERIENCE

Cancer induction is the most important somatic effect of low-dose ionizing radiation. In sharp contrast to the case for the hereditary effects of radiation (Chapter 11), risk estimates for leukemogenesis and carcinogenesis do not rely on animal data but can be based on experience in humans. There is a long history of a link between radiation exposure and an elevated incidence of cancer. Figure 10.1 is a beautiful picture of Marie Curie and her daughter Irene, who both are thought to have died of leukemia as a result of the radiation they received in their experiments with radioactivity. Figure 10.2 is a photograph of the hand of a dentist in New York who held films in patients' mouths for many years and who suffered malignant changes as a result. Quantitative data on cancer induction by radiation come from populations irradiated for medical purposes and exposed deliberately or inadver-



**Figure 10.1.** Marie Curie (seated) at work with her daughter Irene. Both are thought to have died of leukemia as a consequence of the radiation exposure they received during their experiments with radioactivity. (Courtesy of the Austrian Radium Institute and the International Atomic Energy Bulletin.)

tently to nuclear weapons. Persons exposed therapeutically received comparatively high doses, and their susceptibility to the effects of radiation might have been influenced by the medical condition for which treatment was being given. Those exposed to  $\gamma$ -rays and neutrons from nuclear weapons represent a wider cross-section in terms of health and also include individuals exposed to lower doses. In both cases dose rates were high and exposure times brief. There are a few groups of exposed persons to whom these generalizations do not apply. Examples include pitchblende and uranium miners who inhaled the radioactive gas radon and its daughter products over a prolonged period of time, patients injected with



**Figure 10.2.** Hand of a dentist who for 35 years held x-ray films in place in patients' mouths. The thumb has been partially amputated. Damaged skin on the fingers has been replaced by grafts. The lesion on the finger is a skin cancer subsequently removed. (Courtesy of Dr. Victor Bond, Brookhaven National Laboratory.)

radium chloride or thorotrast for medical purposes, and persons who ingested radionuclides in painting luminous dials on clocks and watches with a paint containing radium. A large number of workers have been exposed occupationally, but they have so far yielded few useful quantitative data on cancer risk estimates, except for miners exposed to radon that have resulted in useful quantitative data.

The early human experience of radiation-induced cancer may be summarized as follows:

1. Skin cancer was common in early x-ray workers, principally physicists and engineers who worked around accelerators before radiation-safety standards were introduced.
2. Lung cancer was found to be a frequent problem in pitchblende miners in Saxony, who dug out the ore from which radium was extracted. In later years, lung cancer also was noted in uranium miners in the central Colorado plateau. In both cases

the mines were poorly ventilated and there was a build-up of radon gas in the atmosphere of the mine; radon and its daughter products were breathed in by the miners, depositing atoms of radioactive material in their lungs. The intense local  $\alpha$ -radiation was responsible for inducing lung tumors.

3. Bone tumors were observed in the radium dial painters. The painters were mostly young women who worked in factories in which the luminous dials on clocks and watches were painted with a special paint preparation containing a radioactive material. The workers dipped their brushes into the radium paint and used their tongues to shape the brushes into sharp points to paint the small dials on watches. As a result, some radium was ingested, which, because it is in the same group of the periodic table as calcium, was deposited in the tips of the growing bones. The intense  $\alpha$ -irradiation produced bone tumors. There is also history of bone tumors in persons who, in the 1920s and 1930s, received injections of radium salts for the treatment of tuberculosis or ankylosing spondylitis.
4. An excess incidence of liver tumors was reported in patients in whom the contrast material Thorotrast was used. This contains radioactive thorium, which, if deposited in the liver, produced a small incidence of liver tumors by  $\alpha$ -irradiation.

These early examples are interesting but largely anecdotal. None of these examples involved situations that now constitute a public health hazard; these problems will never happen again, and the dosimetry in each instance is so uncertain that it is rarely possible to deduce any quantitative relationship between the dose of radiation involved and the tumor incidence.

More recent examples of the human experience of radiation-induced cancer and leukemia include the following:

1. The Japanese survivors of the atomic-bomb attacks on Hiroshima and Nagasaki

are the most important single group studied because of their large number, the care with which they have been followed, and the fact that persons of all ages and both sexes received a wide range of doses. About 120,000 persons have been followed carefully, of whom about 50,000 received doses in excess of 0.005Sv. By 1990, there had been 6,000 deaths from cancer, of which about 400 were considered to be an excess mortality caused by radiation. The weapons used on the two cities were very different. The one used on Nagasaki was of a type that would be expected to emit  $\alpha$ -rays with few neutrons and had been previously tested, so dosimetry is based partly on measurements. The weapon used at Hiroshima was of a type never tested before or since, so that dose estimates are based largely on computer simulations. The radiation from this weapon was a mixture of neutrons and  $\gamma$ -rays. In 1986, the dosimetry relating to the atomic bombs was revised. Computer simulations indicated that the proportion of neutrons, especially at Hiroshima, was lower than previously thought, and the  $\gamma$ -ray doses at large distances were higher. The net effect was to increase cancer risk estimates substantially. The United Nations Scientific Committee on Effects of Atomic Radiation (UNSCEAR) report in 1988 and the report of the Committee on Biologic Effects of Ionizing Radiation (BEIR V) in 1990 summarized the new estimates. The numeric values are discussed in this chapter.

2. In Britain, from 1935 through 1944, some 14,000 patients suffering from ankylosing spondylitis were given radiotherapy to various regions of their spine to relieve pain. A small risk of leukemia mortality has been reported in these persons. Although the spondylitic series provides one of the largest bodies of data on leukemia in humans after exposure to  $x$ - or  $\gamma$ -radiation, and the dosimetry is quite good, it is far from ideal, because it lacks a proper control, consisting of patients with the same disease who did not receive  $x$ -ray therapy but whose treatment was otherwise the same. A possible contribution of carcinogenic drugs to the tumor incidence also has been suggested.
3. There is also allegedly an elevated incidence of leukemia in radiologists who joined learned societies before about 1922, before the introduction of radiation safety standards.
4. Thyroid cancer has been observed in children who received radiotherapy for what was thought to be an enlarged thymus. The thyroid was included in the treatment field, and both malignant and benign thyroid tumors have been observed. Breast cancer is also elevated in these individuals.
5. As recently as the 1950s, it was common practice to use  $x$ -rays to epilate children suffering from tinea capitis (ringworm of the scalp). Thyroid cancer from this practice was first reported by Modan and his colleagues in Israel, who treated a large number of immigrant children from North Africa in whom ringworm of the scalp reached epidemic proportions. There is also a significantly increased risk of brain tumors, salivary gland tumors, skin cancer, and leukemia mortality. A comparable group of children in New York for whom  $x$ -rays were used for epilation before the treatment of tinea capitis show quite different results. There were two malignant thyroid tumors, in addition to some benign tumors. There is, however, an incidence of skin cancer around the face and scalp in those areas also subject to sunlight. The skin tumors arose only in white children, and there were no tumors in black children in the New York series.
6. Patients with tuberculosis, who were fluoroscoped many hundreds of times during artificial pneumothorax, have an elevated incidence of breast cancer. This was first reported in Nova Scotia, but the report has been confirmed by a similar

study in New England. The doses are uncertain but must have been 0.8 to 0.9 Gy, because some of the women involved had skin changes in the chest wall on the side frequently fluoroscoped. Patients who received radiotherapy for postpartum mastitis also show an excess incidence of breast cancer.

### CARCINOGENESIS IN LABORATORY ANIMALS

Because so many human data are available for radiation carcinogenesis, the only reason to discuss animal data is to illustrate the shape of the dose-response relationship over a wide range of doses. Figure 10.3 shows the results of a typical experiment in which the incidence of leukemia production is plotted against the dose of radiation absorbed; the incidence of malignancy increases with dose up to a maximum, which usually occurs between 3 and 10 Gy (300-1,000 rad), followed by a decrease with further increases in dose.

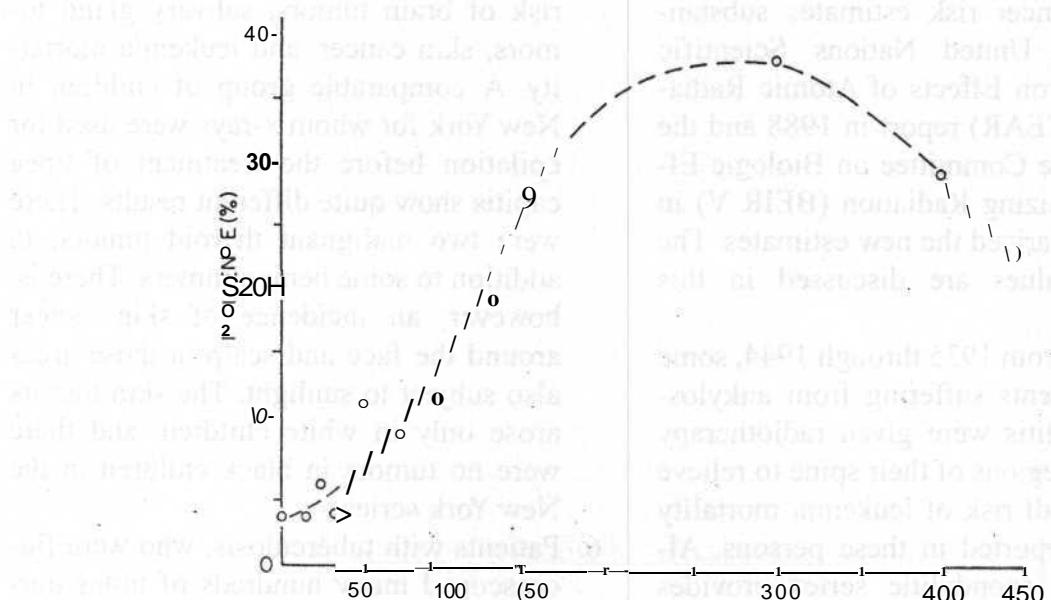
The usual interpretation of such a shape postulates the concurrent presence of two different phenomena: a dose-related increase of

the proportion of normal cells that are transformed into malignant ones and a dose-related decrease of the probability that such cells may survive the radiation exposure. Both of these phenomena normally are operating in the region of doses for which data are available, but to a different degree for various doses and different types of cancer. With this interpretation some of the cells that would otherwise show transformation are killed, so that the fraction actually seen as transformed is reduced at high doses.

It is important to note that tumor incidence does not necessarily continue to increase indefinitely with increasing total-body dose. A qualitatively similar pattern has been observed for a number of tumors in different animals, and there is a clear indication of this trend, too, in the data from the Japanese atomic-bomb survivors.

### THE LATENT PERIOD

The time interval between irradiation and the appearance of a malignancy is known as the **latent period**.



**Figure 10.3.** Incidence of myeloid leukemia in RF male mice exposed to total-body x-irradiation. (From Upton AC: The dose-response relation in radiation-induced cancer. *Cancer Res* 21:717-729, 1961, with permission.)

Leukemia has the shortest latent period. Excess cases began to appear in the survivors of Hiroshima and Nagasaki a few years after irradiation and reached a peak by 7 to 12 years; most cases occurred in the first 15 years. Solid tumors show a longer latency than the leukemias, of anything from 10 to 50 years. For example, an excess incidence of solid tumors is still evident in Japanese survivors exposed to radiation from the atomic bombs in 1945. Indeed, for solid cancers, the excess risk is apparently more like a lifelong elevation of the natural age-specific cancer risk.

As the Japanese data have matured, the concept of a fixed time interval between irradiation and the appearance of the malignancy has been replaced by the concept of "age at expression." Regardless of the age at the time of exposure, radiation-induced tumors tend to be expressed later in life, at the same time as spontaneous tumors of the same type. Breast cancer in women is a good example. This suggests that although radiation may initiate the carcinogenic process at a young age, additional steps are required later in life, some of which may well be hormone-dependent.

### ASSESSING THE RISK

Most data on carcinogenesis in humans involve relatively small numbers of persons who received relatively large doses of radiation. To use the available data to estimate risks as a function of dose, it is necessary to fit the data to a model. There are several reasons for this:

1. Data obtained at relatively high doses must be extrapolated to the low doses of public health concern.
2. No large human population exposed to radiation has yet been studied for its full life span, and so estimates must be projected into the future.
3. The best data pertain to the Japanese irradiated by the atomic bombs, and risk es-

timates based on this must be transferred to other populations that have quite different characteristics, including their natural cancer incidence.

There are two types of models that are conceptually quite different. First, the **absolute risk model** assumes that radiation induces a "crop" of cancers over and above the natural incidence and unrelated to it. This model was used widely in the past. Second, the **relative risk model** assumes that the effect of radiation is to increase the natural incidence *at all ages* subsequent to exposure by a given factor. Because the natural or spontaneous cancer incidence rises significantly in old age, the relative risk model predicts a large number of radiation-induced cancers in old age, also.

The model favored for the most recent assessment of the cancer risks from the Japanese atomic-bomb survivors is a **time-dependent relative risk model**. The excess incidence of cancer was assumed to be a function of dose, the square of the dose, age at exposure, and time since exposure. For some tumors, sex must be added as a variable, for example, in the case of breast cancer.

### COMMITTEES CONCERNED WITH RISK ESTIMATES AND RADIATION PROTECTION

There are two series of reports that analyze available data and come up with risk estimates for radiation-induced cancer. The first is the UNSCEAR reports. This committee reports to the General Assembly at regular intervals; the most recent report appeared in 1988. The second is the committee of the U.S. National Academy of Sciences known as the Committee on the Biological Effects of Ionizing Radiations. Reports appeared periodically, the most recent (BEIR V) appearing in 1990. To a large extent these are "scholarly" committees, inasmuch as they are under no compulsion to draw conclusions if data are not available.

On the other hand, there are committees involved with radiation protection that cannot afford to be scholarly, because they must make recommendations whether or not adequate data are available. First, there is the International Commission on Radiological Protection (ICRP). This commission originally was set up and funded by the first International Congress of Radiology. Over the years, the funding base of this commission has broadened, and it has assumed the role of an independent self-propagating committee. At a national level in the United States, there is the National Council on Radiological Protection and Measurements (NCRP). This is an independent body chartered by Congress and funded from industry, government grants, and professional societies. The NCRP formulates policies for radiation protection in the United States, often but not always following the lead of the ICRP. The recommendations of the NCRP carry no weight in law but are almost always adopted and enforced by the regulatory agencies.

### LEUKEMIA

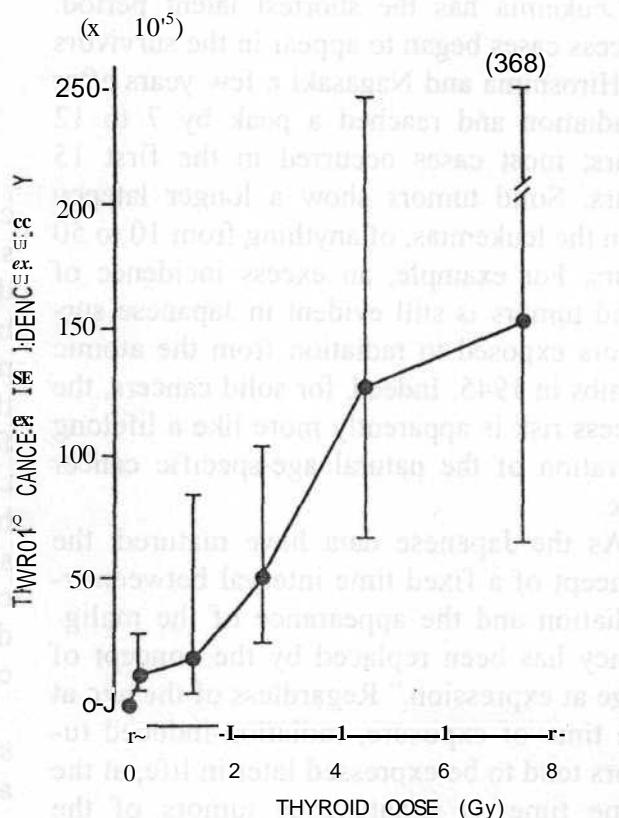
The incidence of chronic lymphocytic leukemia does not appear to be affected by radiation. Acute and chronic myeloid leukemia are the types chiefly responsible for the excess incidence observed in irradiated adults; susceptibility to acute lymphatic, or stem-cell, leukemia seems to be highest in childhood and to decrease sharply during maturation.

Two principal population groups are available for study:

1. Survivors of the atomic-bomb attacks on Hiroshima and Nagasaki
2. Patients treated for ankylosing spondylitis

### THYROID CANCER

The thyroid gland is an organ of high sensitivity for radiation carcinogenesis. This ap-



**Figure 10.4.** Thyroid cancer incidence per person-year (PY) as a function of the radiation dose in the thyroid. Rates adjusted for sex, ethnicity, and interval after irradiation. Error bars represent 90% confidence limits. (From Shore RE, Woodard E, Hildreth N, et al.: JNCI 74: 1177-1184, 1985, with permission.)

pears to be true in children and young people, though in adults radiation is much less efficient in inducing thyroid cancer. The malignant tumors that have been produced, however, consistently have been of a histologically well-differentiated type, which develops slowly and often can be removed completely by surgery or treated successfully with radioactive iodine if metastasized; consequently, these tumors show a low mortality rate. It is estimated that about 5% of those with radiation-induced thyroid cancer die as a result.

The principal population groups available for the derivation of risk estimates for thyroid cancer are as follows:

1. Survivors of the atomic-bomb attacks on Hiroshima and Nagasaki
2. Residents of the Marshall Islands exposed to external radiation and ingested iodine-131 from fallout after the 1954 testing of a thermonuclear device, in whom there was a high incidence of nodule formation and some thyroid cancer (benign as well as malignant tumors)
3. Individuals who ingested radioactive iodine as a result of the Chernobyl accident; this experience shows how very sensitive children are, but that adults are relatively resistant
4. Children treated with x-rays for an enlarged thymus
5. Children treated for diseases of the tonsils and nasopharynx
6. Children epilated with x-rays for the treatment of *tinea capitis*
7. Children treated for cancer

Figure 10.4 shows the incidence of thyroid cancer per person-year as a function of thyroid dose, from the New York series of children *treated for enlarged thymus*.

## BREAST CANCER

Breast cancer may be induced with relatively high frequency by radiation. The cancer is of the type arising initially from duct cells but commonly is found to infiltrate breast tissue.

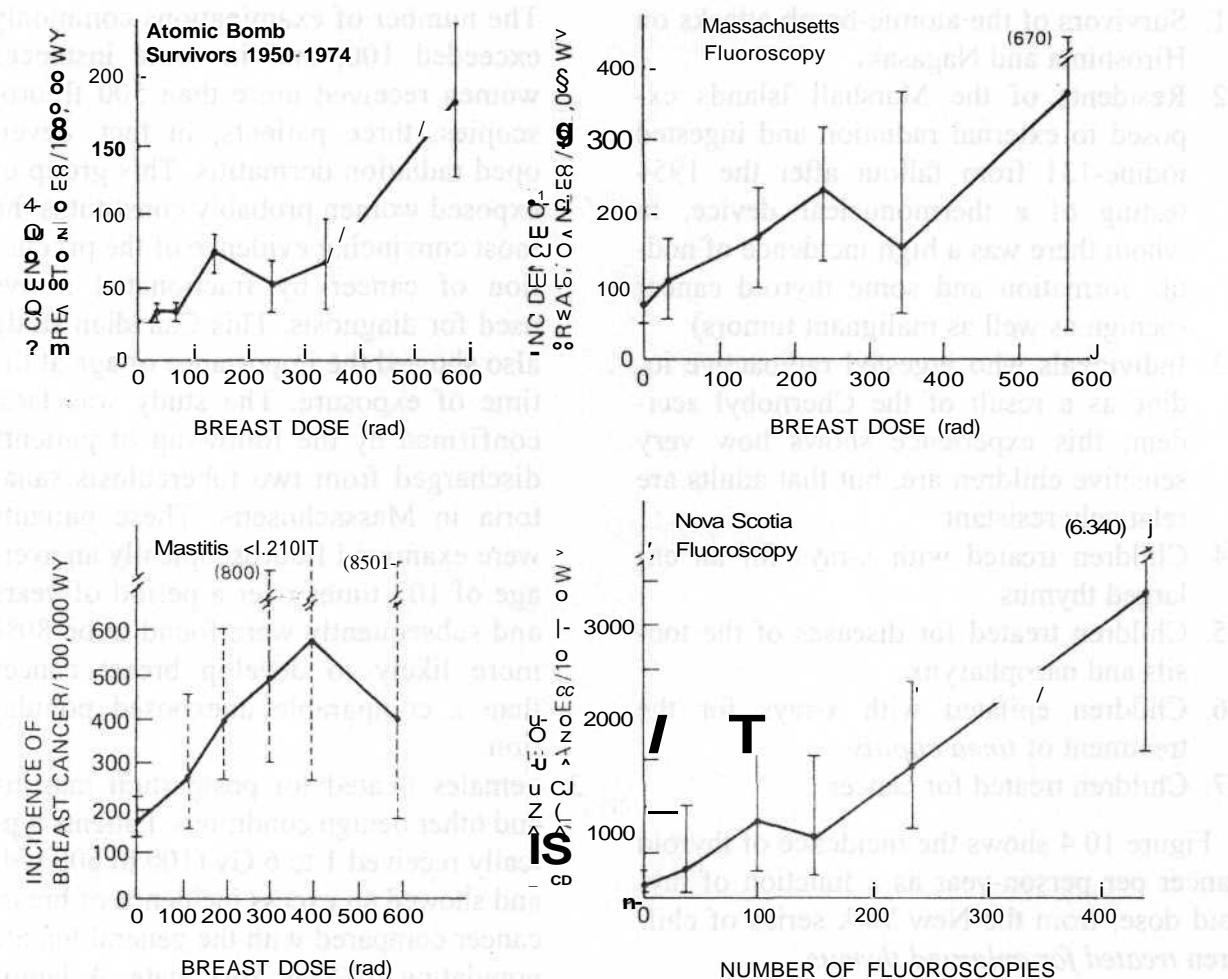
There are three principal exposed populations from which the risk of breast cancer incidence may be derived:

1. Japanese female survivors of the atomic-bomb attacks on Hiroshima and Nagasaki.
2. Female patients in a Nova Scotia sanatorium subjected to multiple fluoroscopies during artificial pneumothorax for pulmonary tuberculosis. There is doubt about the dosimetry, but the dose to breast tissue per fluoroscopy is estimated to be 0.04 to 0.2 Gy (4-20 rad).

The number of examinations commonly exceeded 100, and in some instances women received more than 500 fluoroscopies; three patients, in fact, developed radiation dermatitis. This group of exposed women probably constitutes the most convincing evidence of the production of cancer by fractionated x-rays used for diagnosis. This Canadian study also showed the importance of age at the time of exposure. The study was later confirmed by the follow-up of patients discharged from two tuberculosis sanatoria in Massachusetts. These patients were examined fluoroscopically an average of 102 times over a period of years and subsequently were found to be 80% more likely to develop breast cancer than a comparable unexposed population.

3. Females treated for postpartum mastitis and other benign conditions. Patients typically received 1 to 6 Gy (100 to 600 rad) and showed an excess incidence of breast cancer compared with the general female population of New York state. A legitimate objection to the use of these data for risk estimates is the uncertainty as to whether postpartum mastitis predisposes to breast cancer.

The data for excess incidence of breast cancer in these populations are shown in Figure 10.5. A number of interesting points are immediately apparent. First, the data from the New York series of postpartum mastitis patients are so poor that they do not give any clue as to the shape of the dose-response relationship. Second, there is marked difference in the natural incidence of breast cancer in Japanese women, in whom it is low, compared with American and Canadian women, in whom it is high; nevertheless, in all cases incidence rises with radiation dose. Third, the data for breast cancer are reasonably well fitted by a straight line, giving more credence to the linear extrapolation than any of the other types of cancer.



**Figure 10.5.** incidence of breast cancer as a function of dose for four human populations that allow risk estimates to be made. Note that the natural incidence of breast cancer is low in Japanese women and high in American and Canadian women. (From Boice JD Jr, Land CE, Shore RE, Norman JE, Tokunaga M: Risk of breast cancer following low-dose exposure. Radiology 131:589-597, 1979, with permission.)

### LUNG CANCER

Radiation is but one of a long list of carcinogens for lung cancer: Cigarette smoking, asbestos, chromium salts, mustard gas, hematite, and asphalt derivatives have been implicated, also. Risk estimates come from two principal sources:

1. Persons exposed to external sources of radiation, including the Japanese survivors and those **with** the ankylosing spondylitis. An excess was found even when smoking was taken into account.
2. Underground miners exposed to radon in the mine atmosphere. The naturally occurring deposits of radioactive materials

in the rocks of the earth decay through a long series of steps until they reach a stable isotope of lead. One of these steps involves radon, which, unlike the other elements in the decay series, is a gas. In the closed environment of a mine, workers breathe in radon gas, and some radon atoms decay to the next member of the radioactive series, a solid, which consequently is deposited on the bronchial epithelium. Subsequent steps in the radioactive decay series take place in the lungs, causing intense  $\alpha$ -irradiation of localized surrounding tissue.

There is a clear excess of lung cancer among workers in the uranium mines of the

Colorado plateau in the United States, the uranium mines in Czechoslovakia, nonuranium mines in Sweden, and fluorspar mines in Newfoundland. It remains difficult to separate adequately the contributory effects of radon and cigarette smoking in causing the cancers, because there are too few nonsmoking miners to form an adequate control group. The average duration of exposure usually spans 15 to 20 years, during which standards of safety and ventilation have changed substantially. In any case it is no easy matter to estimate the dose to the critical cells in the basal layer of the epithelium of the lung from a knowledge of the radon concentration in the air that is breathed. There is also some evidence, summarized in the BEIR VI report, of an excess of lung cancer from domestic radon exposure.

## BONE CANCER

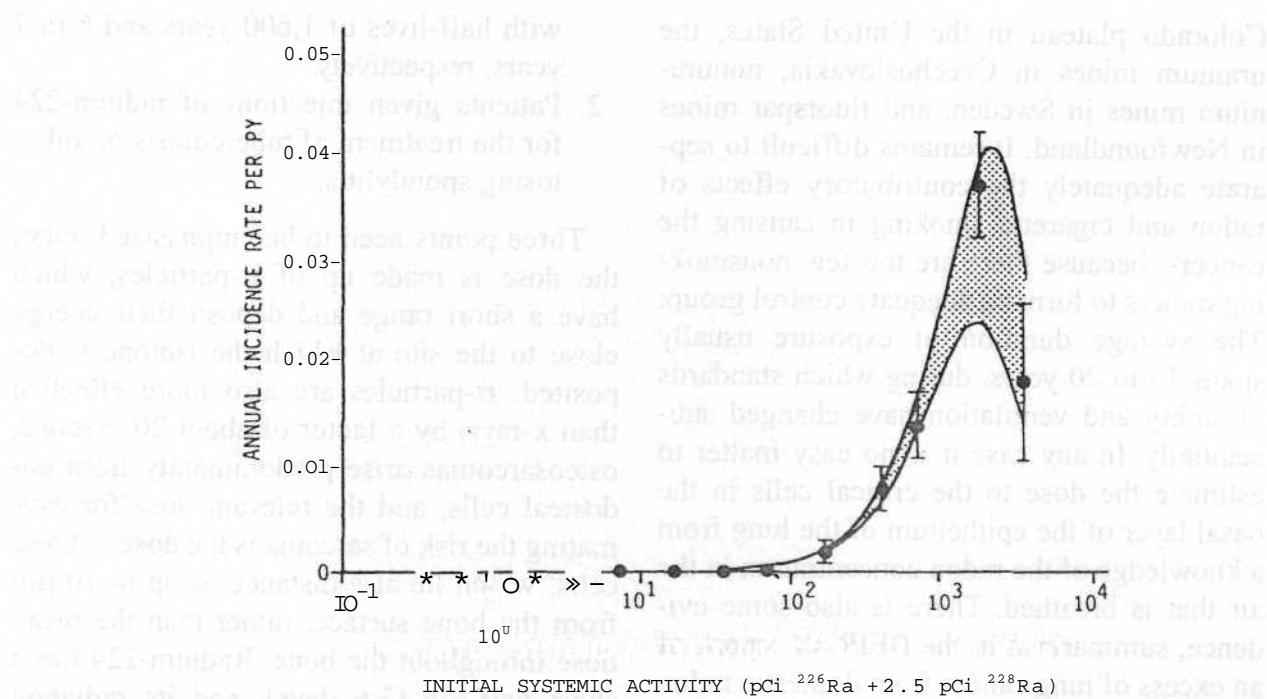
There is some evidence of bone cancer induced by external x-irradiation in children epilated for the treatment of *tinea capitis* and in patients treated for ankylosing spondylitis. The numbers are small and the risk estimates poor. The largest body of data comes from two populations, each of which ingested isotopes of radium that emit high linear energy transfer  $\alpha$ -particles and that follow the metabolic pathways of calcium in the body to become deposited in the bone. The populations include:

1. Young persons, mostly women, employed as dial painters, who ingested radium as a result of licking their brushes into a sharp point for application of luminous paint to watches and clocks. In this group there have been bone sarcomas and carcinomas of epithelial cells lining the paranasal sinuses and nasopharynx. None of these tumors occurred at doses below 5 Gy (500 rad); above this level, the incidence rose sharply, particularly the sarcomas. The radium in these paints consisted of the isotopes radium-226 and radium-228,

with half-lives of 1,600 years and 6 to 7 years, respectively.

2. Patients given injections of radium-224 for the treatment of tuberculosis or ankylosing spondylitis.

Three points need to be emphasized. First, the dose is made up of  $\alpha$ -particles, which have a short range and deposit their energy close to the site at which the isotope is deposited;  $\alpha$ -particles are also more effective than x-rays, by a factor of about 20. Second, osteosarcomas arise predominantly from endosteal cells, and the relevant dose for estimating the risk of sarcoma is the dose to these cells, which lie at a distance of up to 10  $\mu\text{m}$  from the bone surface, rather than the mean dose throughout the bone. Radium-224 has a short half-life (3.6 days), and its radiation therefore largely is delivered while it is still present on the bone surface. This contrasts sharply with radium-226 and **radium-228**, which have long half-lives and consequently become distributed throughout bone during their periods of radioactive decay. The dose to endosteal cells from radium-224 is about nine times larger than the dose averaged throughout bone, whereas it is about two thirds of the mean bone value from radium-226. Consequently, it is difficult to compare data from the two groups of persons who ingested these very different isotopes of radium. Third, age at the time of exposure is an important factor in the development of bone cancer. For young persons and possibly also for those exposed *in utero*, the rapid deposition of bone-seeking radioisotopes during active bone growth might confer a higher risk of cancer than in adults. There is, in general, poor agreement among the risk estimates derived from the various groups of persons showing an excess of bone cancer, so that risk estimates must be very crude. Figure 10.6 shows the incidence of bone sarcoma in female dial painters as a function of activity of radium ingested. These data imply that a linear extrapolation from high to low doses would overestimate risks at low doses. It appears that sarcomas are induced only after doses that are sufficient to



**Figure 10.6.** A semilogarithmic plot of bone-sarcoma incidence rate as a function of systemic intake for female dial painters employed before 1950, showing a dose-squared exponential fit. The shaded band indicates the range covered by the fitted function if the coefficients are allowed to vary by  $\pm 1$  standard deviation. (From Rowland R, Stehner AF, Lucas HF: Health Phys 44:15-31, 1983, with permission.)

cause tissue damage and therefore to stimulate cell proliferation.

## SKIN CANCER

The first neoplasm attributed to x-rays was an epidermoid carcinoma on the hand of a radiologist, which was reported in 1902. In the years that followed, several hundred such cases arose among physicians, dentists, physicists, and x-ray technicians, in an era in which safety standards were virtually nonexistent. In most cases the onset of neoplasms followed chronic radiodermatitis and a long latent period. Squamous cell and basal cell carcinomas have been most frequently observed, and occasionally a sarcoma of the subcutaneous tissues has been seen. Since the evolution of modern safety standards, epidermoid carcinoma has ceased to be an occupational disease of radiation workers.

Radiation-induced skin cancers are diagnosed readily and treated at an early stage of development, and there is a large difference between rates of incidence and mortality.

There is a small excess incidence of skin cancer in the children epilated with x-rays for the treatment of tinea capitis.

## QUANTITATIVE RISK ESTIMATES FOR RADIATION-INDUCED CANCER

Despite a diverse collection of data for cancer in humans from medical sources, both the BEIR V and the latest UNSCEAR reports elected to base their risk estimates almost entirely on the data from the survivors of the atomic-bomb attacks on Hiroshima and Nagasaki.

Table 10.1 shows a summary of the data for mortality from solid tumors and leukemias in the atomic-bomb survivors for the years 1950 through 1990. The raw data are shown principally to emphasize the relative poverty of the data; only a few hundred excess cancer deaths caused by radiation are involved, compared with many thousands of deaths from naturally occurring malignancies—and these must be allocated to different dose groups and different sites. It is estimated that by the time an irradiated

**TABLE 10.1.** Observed and Expected Deaths for Solid Cancers and Leukemia, 1950-1990

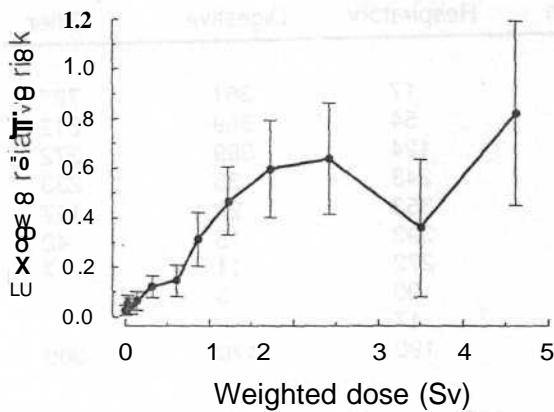
Dose, Sv	Subjects	Observed Deaths	Expected Background	Excess Deaths
<b>Solid Cancers</b>				
0 (<0.005)	36,459	3,013	3,055	-42
0.005-0.1	32,849	2,795	2,710	85
0.1-0.2	5,467	504	486	18
0.2-0.5	6,308	632	555	77
0.5-1.0	3,202	336	263	73
1.0-2.0	1,608	215	131	84
>2.0	679	83	44	39
Total	86,572	7,578	7,244	334
<b>Leukemia</b>				
0 (<0.005)	35,458	73	64	9
0.005-0.1	32,915	59	62	-3
0.1-0.2	5,613	11	11	0
0.2-0.5	6,342	27	12	15
0.5-1.0	3,425	23	27	16
1.0-2.0	1,914	26	4	22
>2.0	905	30	2	28
Total	86,572	249	162	87

Adapted from Pierce CA, Shimizu Y, Preston DL, Vaeth M, Mabuchi K: Studies of the mortality of atomic bomb survivors: Report 12, Part I. Cancer: 1950-1990. Radiat Res 146:1-27, 1996, with permission.

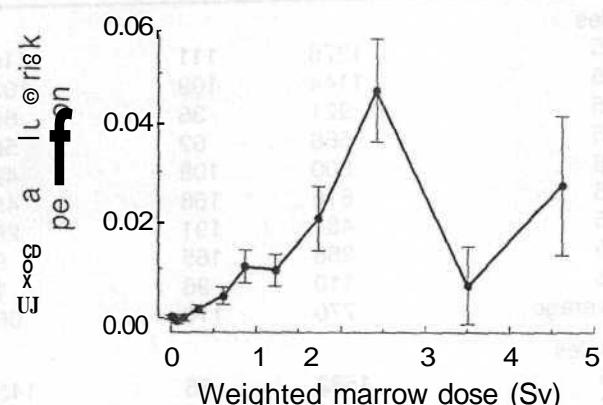
ated population has lived out its entire life-span, the ratio of radiation-induced solid cancers to leukemias will be in the range 4 to 6.

Figure 10.7 shows the dose-response data for solid tumors, which are quite linear up to about 3 Sv but flatten out significantly at

higher doses, because of cell killing. Figure 10.8 shows the dose-response data for leukemia. At the lower doses, at which most cases occurred, the excess risk is not a linear function of dose, but it is best fitted by a linear-quadratic relationship.



**Figure 10.7.** Data for solid cancers in the atomic-bomb survivors, 1950 through 1990, shown in terms of the excess relative risk (ERR) as a function of dose. The ERR appears to be quite linear for doses below 3 Sv but flattens off significantly at higher doses, probably because of cell killing. (Adapted from Pierce DA, Shimizu Y, Preston DL, Vaeth M, Mabuchi K: Studies of the mortality of atomic bomb survivors: Report 12, Part I. Cancer: 1950-1990. Radiat Res 146:1-27, 1996, with permission.)



**Figure 10.8.** Data for leukemia in the A-bomb survivors, shown in terms of the excess absolute risk as a function of dose. Over the first few sieverts, there is a departure from linearity; a linear-quadratic function of dose is a better fit. (Adapted from Pierce DA, Shimizu Y, Preston DL, Vaeth M, Mabuchi K: Studies of the mortality of atomic bomb survivors: Report 12, Part I. Cancer: 1950-1990. Radiat Res 146:1-27, 1996, with permission.)

**TABLE 10.2.** Excess Cancer Mortality: Lifetime Risk Per 100,000 at 0.1 Sv

	BEIRV (US Population)		UNSCEAR 88 (Japanese Population)	
	Males	Females		
Breast		70	Breast	60
Respiratory	190	150	Lung	151
Digestive system	170	290	Stomach	126
Other solid	300	220	Colon	79
Leukemia	110	80	Other solid	194
Total	770	810	Leukemia	100
			Total	710

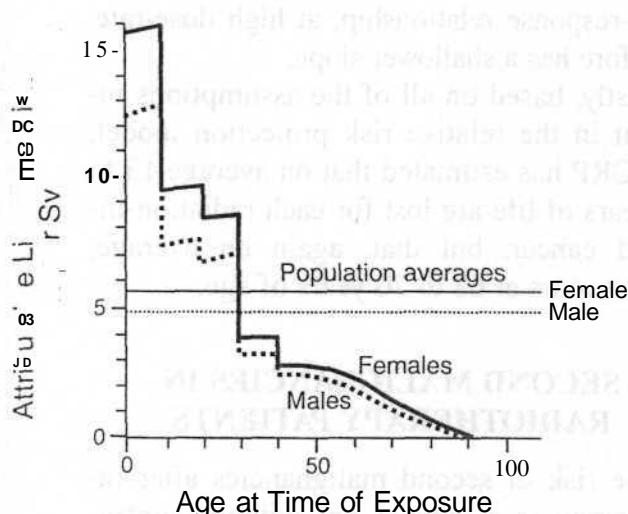
The BEIRV and UNSCEAR committees analyzed the data in more detail and considered a number of specific cancer sites rather than lumping together all solid cancers. Table 10.2 summarizes these data. The two committees considered slightly different groupings of cancer sites, but in general the agreement between the two is more remarkable than the differences. The risk estimates for these principle tumor types that have been shown to be radiogenic differ only by a factor of 2 or 3. It should be noted that cancer risks in other organs or tissues are much lower or too low to be detectable.

As the data from Japan have matured, and more detailed information has become available, it is evident that the risk of radiation-induced cancer varies considerably with age at the time of exposure. Table 10.3 summarizes excess deaths from several types of cancer in males and females, as a function of age at time of exposure. In most cases those exposed at an early age are much more susceptible than at later times. The difference is most dramatic for thyroid cancer; children are very radiosensitive, while adults are quite resistant. It is also dramatic for breast cancer in fe-

**TABLE 10.3.** Cancer Excess Mortality by Age at Exposure and Site for 100,000 Persons of Each Age Exposed to 0.1 Sv (10 rem)

Age at Exposure, y	Total	Leukemia	Nonleukemia	Respiratory	Digestive	Other
<b>Males</b>						
5	1276	111	1165	17	361	787
15	1144	109	1035	54	369	612
25	921	36	885	124	389	372
35	566	62	504	243	28	233
45	600	108	492	353	22	117
55	616	166	450	393	15	42
65	481	191	290	272	11	7
75	258	165	93	90	5	
85	110	96	14	17		
Average	770	110	660	190	170	300
<b>Females</b>						
5	1532	75	1457	129	48	625
15	1566	72	1494	295	70	653
25	1178	29	1149	52	125	293
35	557	46	511	43	208	187
45	541	73	468	20	277	71
55	505	117	388	6	273	64
65	386	146	240		172	52
75	227	127	100		72	26
85	90	73	17		15	4
Average	810	80	730	70	150	220

Adapted from Committee on the Biological Effects of Ionizing Radiation: Health Effects of Exposure to Low Levels of Ionizing Radiation. Washington, DC, National Academy of Sciences/National Resource Council, 1990, with permission.



**Figure 10.9.** The attributable lifetime risk from a single small dose of radiation at various ages at the time of exposure. Note the dramatic decrease in radiosensitivity with age. The higher risk for the younger age groups is not expressed until late in life. These estimates are based on a multiplicative model and on a dose and dose-rate effectiveness factor (DDREF) of two. (Adapted from ICRP Publication 60: Oxford, Pergamon Press, 1990)

males; females exposed before 15 years of age are most susceptible; women 50 years of age or older show little or no excess. There are exceptions to this general rule. Susceptibility to radiation-induced leukemia is relatively constant throughout life, and susceptibility to respiratory cancers increases in middle age. The overall risk, however, drops dramatically with age; children and young adults are much more susceptible to radiation-induced cancer than the middle-aged and old. This is illustrated dramatically in Figure 10.9.

### DOSE AND DOSE-RATE EFFECTIVENESS FACTOR

The Japanese data relate only to high doses and high dose rates, because they are based on the atomic-bomb survivors. Both the UNSCEAR 88 and BEIR V committees considered that there is a dose-rate effect for low linear energy transfer radiations; that is, fewer malignancies are induced if a given dose is spread out over a period of time at low dose

rate than if it is delivered in an acute exposure. There are insufficient data, however, in the human to be certain of a quantitative value for the magnitude of the dose-rate effect; consequently, a range of 2 to 10 was proposed, based on animal data. For purposes of radiation protection, the ICRP recommends a dose and dose-rate effectiveness factor of 2 for doses below 0.2 Gy (20 rad) at any dose rate and higher doses if the dose rate is less than 0.1 Gy/h (10 rad/h).

### SUMMARY OF RISK ESTIMATES

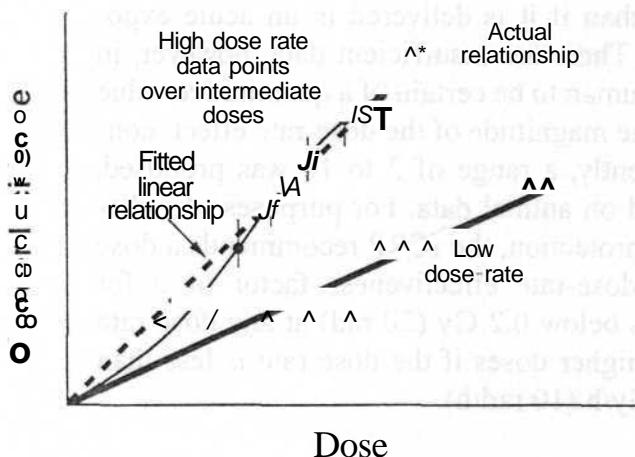
In summarizing all of these risk estimates for the practical purposes of radiation protection, the ICRP recommends the following figures, listed in Table 10.4. For a working population composed of both sexes, the lifetime risk of fatality from cancer is  $8 \times 10^{-2}$  per sievert for high doses and dose rates, and  $4 \times 10^{-2}$  per sievert for low doses and low dose rates. The comparable values for the whole population are a little higher because of the sensitivity of the young at  $10 \times 10^{-2}$  per sievert for high doses and dose rates, and  $5 \times 10^{-2}$  per sievert for low doses and dose rates. The overall philosophy of the ICRP, as far as cancer risk estimates is concerned, is illustrated in Figure 10.10.

For acute doses of low linear energy transfer radiation, the most likely form of the relationship between dose to an organ and the probability of an induced cancer is shown by

**TABLE 10.4.** International Commission on Radiological Protection Summary of Risks of Cancer Lethality by Radiation

	High Dose High Dose Rate	Low Dose Low Dose Rate
Working, population	$8 \times 10^{-2}$ per Sv	$4 \times 10^{-2}$ per Sv
Whole population	$10 \times 10^{-2}$ per Sv	$5 \times 10^{-2}$ per Sv

International Commission on Radiological Protection: Recommendations. Annals of the ICRP Publication 60. Oxford, Pergamon Press, 1990, with permission.



**Figure 10.10.** The shapes of dose-response curves for radiation-induced carcinogenesis. For high dose and high dose-rate exposures, the actual relationship is likely to have a complex shape. Excess cancer incidence is a linear-quadratic function of dose and bends over at high doses as cell killing of target cells becomes important. Because human data points are available over a limited dose range (and are of poor quality), they may be fitted adequately by a straight line, that is, a linear function of dose. For exposures at low dose rate, the dose-response curve for excess cancer incidence is shallower and is an extrapolation of the initial region of the high dose-rate curve. (Drawn from the ideas of Dr. Warren Sinclair)

the thin solid line in Figure 10.10, that is, a linear-quadratic relationship at low to intermediate doses, followed by a flattening of the curve and a subsequent decrease with dose as cell killing of some target cells becomes important. A dose-response relationship having this general shape already has been shown to apply for leukemia in mice (Fig. 10.3).

In practice, the data available from the Japanese survivors cover a limited range of doses from 0.005 to 4 Gy (illustrated schematically in Figure 10.10). Both the UNSCEAR 88 and BEIR V committees found that for solid tumors, the excess incidence appeared to be a linear function of dose (shown by the dashed line in Figure 10.10). This is probably a consequence of the limited dose range over which data are available and the bending over of the curve at higher doses. At low dose rates, the dose-response relationship is essentially an extension of the initial portion of the complex

dose-response relationship, at high dose-rate, therefore has a shallower slope.

Lastly, based on all of the assumptions inherent in the relative risk projection model, the ICRP has estimated that on average 13 to 15 years of life are lost for each radiation-induced cancer, but that, again on average, death occurs at 68 to 70 years of age.

## SECOND MALIGNANCIES IN RADIOTHERAPY PATIENTS

The risk of second malignancies after radiotherapy is a subject not without controversy. One of the reasons for the uncertainty is that patients undergoing radiotherapy are often at high risk of a second cancer because of their lifestyles, and this factor is more dominant than the radiation risk.

There are many single-institution studies in the literature involving radiotherapy for a variety of sites that conclude that there was no increase in second malignancies, although a more accurate assessment would have been that the studies had limited statistical power to detect relatively small increased incidence of second malignancies induced by the treatment.

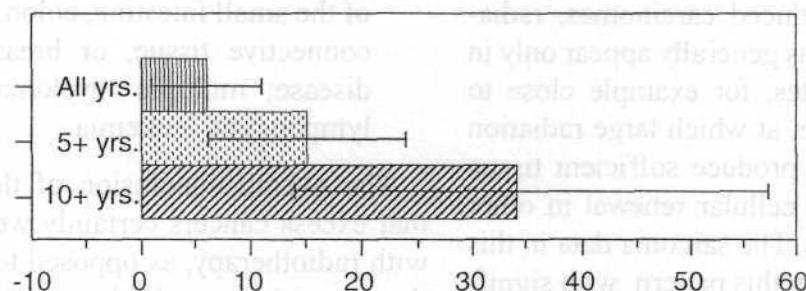
Whenever large studies have been performed, radiotherapy has been shown to be associated with a statistically significant, though very small, enhancement in the risk of second malignancies, particularly in long-term survivors. The three requirements for a study to be credible are:

1. A sufficiently large number of patients
2. A suitable comparison group, that is, patients with the same cancer treated by some means other than radiation
3. A sufficiently long follow-up for radiation-induced solid tumors to become manifest

A number of studies satisfy these criteria; these will be discussed in some detail.

### Second Cancers after Radiotherapy for Prostate Cancer

Brenner and colleagues described a study using data from the National Cancer Insti-

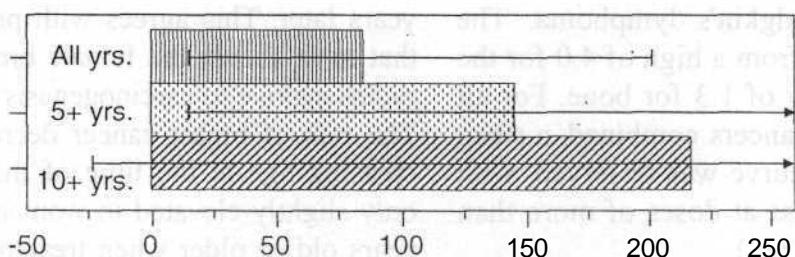


**Figure 10.11.** Percentage increase in relative risk (RR) for all solid tumors (except prostate cancer) for individuals who received radiotherapy for prostate cancer relative to the risk for individuals who underwent surgery for prostate cancer. (Adapted from Brenner DJ, Curtis RE, Hall EJ, Ron E: Second cancers after radiotherapy for prostate cancer. *Cancer* 88, 398-406, 2000, with permission.)

Institute's Surveillance, Epidemiology and End Results (SEER) Program. The SEER program is a set of geographically defined, population-based tumor registries, covering approximately 10% of the U.S. population. The database contained information on 51,584 men with prostate cancer treated by radiotherapy and 70,539 who underwent surgery. There was no evidence of a difference in the risk of leukemia for radiotherapy versus surgery patients, but the risk of a second solid tumor at any time postdiagnosis was significantly greater after radiotherapy than after surgery, by about 6%. This increased risk became greater with time and reached 34% after 10 years or more. The most dramatic increases were for the bladder (77%) and the rectum (105%) for 10 years or more following diagnosis. The relative risks are shown in Figure

10.11. The risk of sarcomas in the heavily irradiated tissues within the field amount to 145% at 5 years or more compared with surgical patients. The relative risks are shown in Figure 10.12.

It is interesting to note that the increase in relative risk for carcinoma of the lung, which was exposed to a relatively low dose (about 0.5 Gy or 50 rad), is of the same order as that for carcinomas of the bladder, rectum, and colon, all of which were subject to much higher doses (typically more than 5 Gy or 500 rad). This pattern may reflect the fact that carcinomas, originating in actively dividing cells or cells under hormonal control, can be efficiently induced by relatively low doses of radiation, as evidenced by the atomic-bomb survivors, but the cancer risk at high doses decreases, because of the effects of cell killing. In contrast to this pat-



**Figure 10.12.** Percentage increase in relative risk (RR) for sarcomas in or near the treatment field for individuals who received radiotherapy for prostate cancer relative to individuals who underwent surgery. Although the number of tumors involved is much smaller than for all solid tumors (shown in Figure 10.10), the relative risks are extremely high. (Adapted from Brenner DJ, Curtis RE, Hall EJ, Ron E: Second cancers after radiotherapy for prostate cancer. *Cancer* 88, 398-406, 2000, with permission.)

tern for radiation-induced carcinomas, radiation-induced sarcomas generally appear only in heavily irradiated sites, for example close to the treatment volume, at which large radiation doses are needed to produce sufficient tissue damage to stimulate cellular renewal in other mostly dormant cells. The sarcoma data in this study appear to follow this pattern, with significant radiation-related risks being exhibited for sites in and close to the treatment volume, but no significant increases being shown for more distant sites.

### Radiation Therapy for Carcinoma of the Cervix

In the largest study of its kind, Boice and colleagues studied the risk of second malignancies in a wide range of organs and tissues as a consequence of the treatment by radiation of carcinoma of the uterine cervix. This huge international study was a "*tour de force*." The paper had 42 authors from 38 institutions representing both sides of the Atlantic. Such a collaboration allowed the accumulation of data from 150,000 patients to be studied. This study is strengthened enormously by the fact that an ideal control group is available for comparison. This malignancy is equally well treated by radiation or surgery. The results can be summarized as follows:

1. Very high doses, on the order of several hundred grays (several tens of thousands of rads), were found to increase the risk of cancer of the bladder, rectum, vagina, possibly bone, uterine corpus, and cecum and of non-Hodgkin's lymphoma. The risk ratios vary from a high of 4.0 for the bladder to a low of 1.3 for bone. For all female genital cancers combined, a steep dose-response curve was observed, with a five-fold excess at doses of more than 150 Gy (15,000 rad).
2. Doses of several grays (several hundred rads) increased the risk of stomach cancer and leukemia.
3. Perhaps surprisingly, radiation was found not to increase the overall risk of cancers

of the small intestine, colon, ovary, vulva, connective tissue, or breast; Hodgkin's disease; multiple myeloma; or chronic lymphocytic leukemia.

The overall conclusion of this study was that excess cancers certainly were associated with radiotherapy, as opposed to surgery, and that the risks were highest among long-term survivors and concentrated among women irradiated at relatively young ages.

### Second Cancers among Long-term Survivors from Hodgkin's Disease

Bhatia and colleagues reported that 17 of 483 girls in whom Hodgkin's disease was diagnosed before the age of 16 years subsequently developed breast cancer, with radiotherapy implicated in the majority of cases. The ratio of observed to expected cases is 75.3. Another study (by Sankila and colleagues) involved 1,641 patients treated for Hodgkin's disease as children in five Nordic countries and reported a relative risk that was 17 times higher than the general population, based on 16 cases of breast cancer. The biggest study of this kind, published by Travis and colleagues, evaluated 3,869 women in population-based registries participating in the SEER program. All these women received radiotherapy as an initial treatment for Hodgkin's disease. Breast cancer developed in a total of 55 patients, which represents a ratio of observed to expected cases of 2.24. The risk of breast cancer, however, was 60.57% in women treated before the age of 16 years, with most tumors appearing 10 or more years later. This agrees with previous studies that have shown the female breast to be very radiosensitive to carcinogenesis at young ages. The risk of breast cancer decreased with increasing age at the time of therapy and was only slightly elevated in women who were 30 years old or older when treated. The most recent paper on this subject (by Nyandoto and colleagues) studied only 202 patients with Hodgkin's disease receiving radiotherapy but found that 27 developed a second cancer, most of which were in or close to the treatment field.

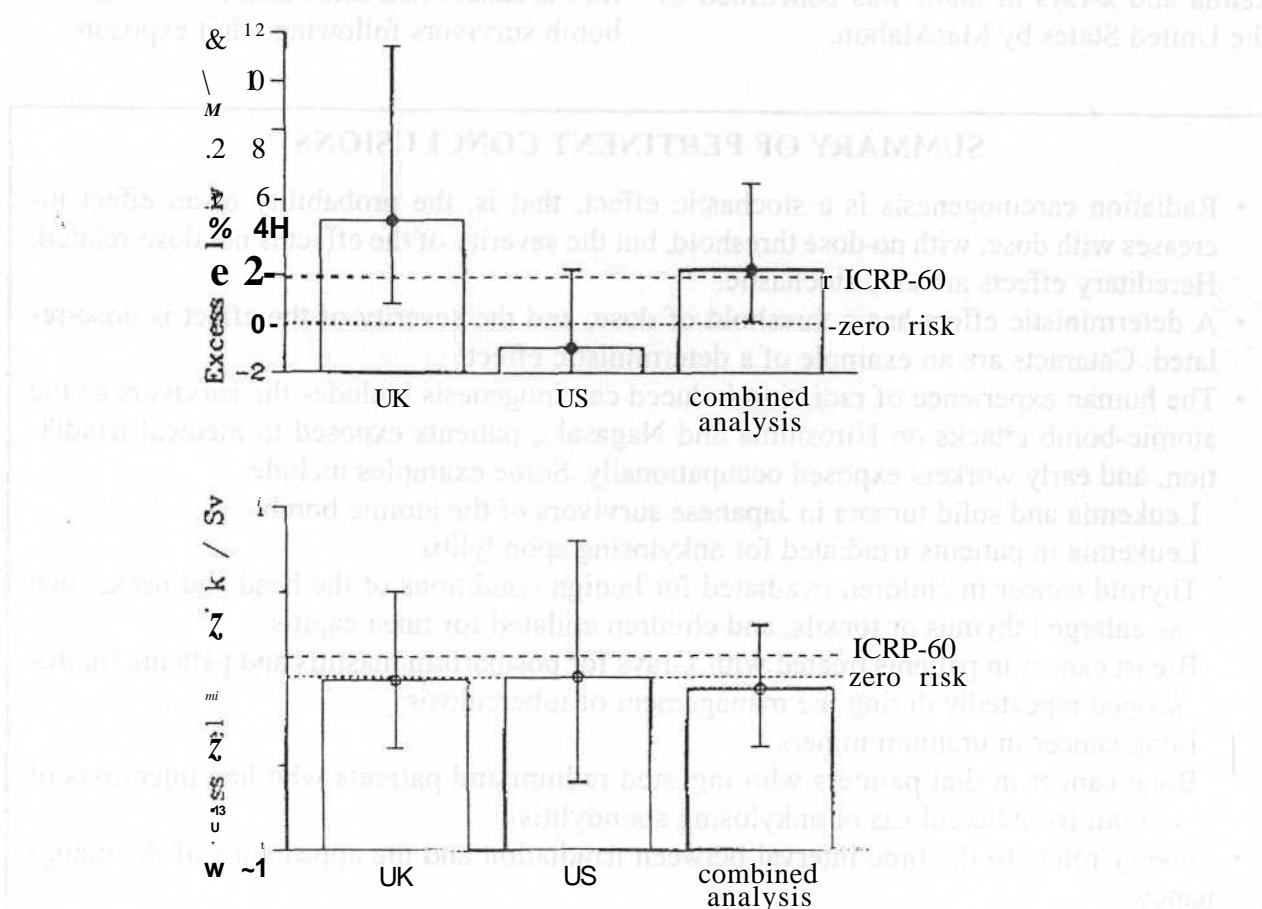
The most common sites for cancers were the lung and breast, but there was a large spectrum of sites, and the risk was greatest in those irradiated at young ages.

These studies clearly show that, if an adequate cohort can be studied, there is a clear excess of second cancers induced by radiotherapy. The data confirm previous studies that show that in the young, the breast is especially sensitive to the carcinogenic effects of radiation. In addition, excess cancers develop with a latency of 10 years or more and persist for decades after exposure.

### CANCER RISKS IN NUCLEAR-INDUSTRY WORKERS

The International Agency for Research on Cancer carried out a huge study involving

over 95,000 nuclear industry workers in the United States, the United Kingdom, and Canada. For all solid cancers combined there was no evidence of an increased risk associated with radiation in any of the three countries. There was a small statistically significant excess of leukemia in U.K. workers. In U.S. workers, there was a deficit in leukemia, the familiar "healthy worker" syndrome previously noted in the United States because in the past radiation workers tended to belong to higher socioeconomic groups and also needed to pass a medical examination to be hired. The excess disappears if the data for all three countries are pooled. Figure 10.13 shows the excess relative risks for leukemia and solid cancers in this study, which have sufficiently large standard errors that they are equally consistent with zero risk, or with the risk esti-



**Figure 10.13.** Excess relative risk for leukemia (**top**) and solid cancers (**below**) from the International Agency for Research on Cancer study. The data are consistent with either zero risk or a risk appropriately extrapolated from the atomic-bomb survivor data (labeled *ICRP-60*, referring to the report which recommends 4% per Sievert as the cancer risk.)

mated by ICRP based on the Japanese atomic-bomb survivors. The study illustrates the futility of trying to detect the possible slight excess of cancer in radiation workers, against the high natural background, even if 95,000 are studied. On the other hand, significant excesses of cancer have been detected in nuclear workers from the former Soviet Union, where much higher doses were involved.

### CHILDHOOD CANCER AFTER RADIATION EXPOSURE *IN UTERO*

In a widely publicized British study, Stewart and her colleagues reported an excess of leukemia and childhood cancer in children irradiated *in utero* as a consequence of diagnostic x-ray examinations involving the pelvis of the mother. An association between leukemia and x-rays *in utero* was confirmed in the United States by MacMahon.

This has been a highly controversial topic. It is discussed in more detail in Chapter 12. In a recent paper, Doll and Wakeford summarized all of the available data and came to the conclusion that radiation was the causative agent. They concluded that:

- Low-dose irradiation of the fetus *in utero*, particularly in the last trimester, causes an increased risk of childhood malignancies.
- An obstetric x-ray examination, even though the dose is only about 10 mGy (1 rad), increases the risk of childhood cancer by 40%.
- The excess absolute risk is about 6% per gray.

The relative risk of 40% is very high because, of course, cancer is relatively rare in children. The absolute risk works out to be about 6% per gray, which is not very different for the cancer risk calculated from the atomic-bomb survivors following adult exposure.

### SUMMARY OF PERTINENT CONCLUSIONS

- Radiation carcinogenesis is a stochastic effect; that is, the probability of an effect increases with dose, with no dose threshold, but the severity of the effect is not dose-related. Hereditary effects are also stochastic.
- A deterministic effect has a threshold of dose, and the severity of the effect is dose-related. Cataracts are an example of a deterministic effect.
- The human experience of radiation-induced carcinogenesis includes the survivors of the atomic-bomb attacks on Hiroshima and Nagasaki, patients exposed to medical irradiation, and early workers exposed occupationally. Some examples include:
  - Leukemia and solid tumors in Japanese survivors of the atomic bomb
  - Leukemia in patients irradiated for ankylosing spondylitis
  - Thyroid cancer in children irradiated for benign conditions of the head and neck, such as enlarged thymus or tonsils, and children epilated for tinea capitis
  - Breast cancer in patients treated with x-rays for postpartum mastitis and patients fluoroscoped repeatedly during the management of tuberculosis
  - Lung cancer in uranium miners
  - Bone cancer in dial painters who ingested radium and patients who had injections of radium for tuberculosis or ankylosing spondylitis
- Latency refers to the time interval between irradiation and the appearance of the malignancy.
- The shortest latency is for leukemia, with a peak of 5-7 years. For solid tumors, the latency may extend for 45 years or more.
- Regardless of the age at exposure, radiation-induced malignancies tend to appear at the same age as spontaneous malignancies of the same type. Indeed, for solid cancers, the ex-

cess risk is apparently more like a lifelong elevation of the natural age-specific cancer risk.

- To determine risk estimates for radiation-induced cancer from observed data (the Japanese atomic-bomb survivors), a model must be assumed because
  1. Data must be projected out to a full lifespan, because no exposed population has yet lived out its lifespan
  2. Data must be extrapolated from high to low doses
  3. Risks must be "transferred" from (for example) a Japanese to Western population with different natural cancer rates.
- There are two principal risk models. The absolute risk model assumes that radiation produced a discrete "crop" of cancers, over and above the spontaneous level and unrelated to the spontaneous level. The relative risk model assumes that radiation increases the spontaneous incidence by a factor. Because the natural cancer incidence increases with age, this model predicts a large number of excess cancers appearing late in life after irradiation.
- The most recent reassessment of radiation-induced cancer risks by the BEIR V committee was based on a time-related relative risk model. Excess cancer deaths were assumed to depend on dose, square of the dose, age at exposure, time since exposure, and, for some cancers, sex.
- For solid tumors, the excess mortality rate was found to be a linear function of dose up to about 3 Sv, with a flattening off at higher doses, presumably because of cell killing.
- Leukemia data were best fitted by a linear-quadratic function of dose.
- The Japanese atomic-bomb data refers to an acute exposure at a high dose rate. A dose and dose-rate effectiveness factor is needed to convert risk estimates to the low dose and low dose rates encountered in radiation protection. From animal studies this is anywhere from 2 to 10. The ICRP assumes a value of 2.
- Based on reports of the UNSCEAR and BEIR V committees, the ICRP suggests a risk estimate of excess cancer mortality in a working population of  $8 \times 10^{-2}$  per sievert for high doses and high dose rates, and  $4 \times 10^{-2}$  per sievert for low doses and low dose rates.
- For the general population, slightly higher risks apply because of the increased susceptibility of the young. The estimates are  $10 \times 10^{-2}$  per sievert for high doses and dose rates and  $5 \times 10^{-2}$  per sievert for low doses and dose rates.
- The ICRP estimates that, on average, 13 to 15 years of life are lost for each radiation-induced cancer and that death occurs at age 68 to 70 years.
- There is a clear excess of second cancers induced by radiation therapy, both in heavily irradiated tissue and in more remote organs. This is evident if a sufficiently large number of patients and an adequate control group are available for study.
- Large studies show a clear excess of second cancers after radiotherapy for prostate cancer, carcinoma of the cervix, and Hodgkin's lymphoma. An excess has also been shown following radiation therapy for breast cancer, carcinoma of the testes, and various childhood malignancies.
- The International Agency for Research on Cancer studied 95,000 workers in the nuclear industry. It is not possible to distinguish between zero risk and the risk extrapolated from the atomic-bomb survivors, 4% per sievert.
- Irradiation *in utero* by diagnostic x-rays appears to increase the spontaneous incidence of leukemia and childhood cancers by a factor of about 1.5. This is a high relative risk because malignancies in children are rare, but the absolute risk is about 6% per Gy—not very different from the risk estimate from the A-bomb survivors for adult exposure.

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## Hereditary Effects of Radiation

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GERM-CELL PRODUCTION IN THE MALE  
AND FEMALE  
REVIEW OF BASIC GENETICS  
MUTATIONS  
RADIATION-PRODUCED HEREDITARY  
EFFECTS  
RELATIVE VERSUS DIRECT (OR  
ABSOLUTE) MUTATION RISK

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RELATIVE MUTATION RISK ASSESSED IN  
THE MEGAMOUSE PROJECT  
HEREDITARY EFFECTS OF RADIATION IN  
HUMANS  
NUMERICAL VALUES OF HEREDITARY  
RISK  
SUMMARY OF PERTINENT CONCLUSIONS

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In the male mammal, spermatozoa arise from the germinal epithelium in the seminiferous tubules of the testes, and their production is continuous from puberty to death. The spermatogonial (stem) cells consist of several different populations that vary in their sensitivity to radiation. The postspennatogonial cells pass through several stages of development: primary spermatocytes, secondary spermatocytes, spermatids, and finally spermatozoa. The division of a spermatogonium to the development of mature sperm involves a period of 6 weeks in the mouse and 10 weeks in the human. The effect of radiation on fertility is not apparent immediately, because the postspermatogonial cells are relatively resistant compared with the sensitive stem cells. After exposure to a moderate dose of radiation, the individual remains fertile as long as mature sperm cells are available, but decreased fertility or even temporary sterility follows if these are used up. The period of sterility lasts until the spermatogonia are able to repopulate by division.

The threshold for temporary sterility in men exposed to a single absorbed dose in the testes is about 0.15 Gy (15 rad). Under pro-

longed . exposure conditions, the threshold dose rate is about 0.4 Gy/y (40 rad/y). The corresponding values for permanent sterility are about 3.5 to 6 Gy (350-600 rad) and 2 Gy/y (200 rad/y). The induction of sterility by radiation in human males does not produce significant changes in hormone balance, libido, or physical capability.

The production of mature germ cells in the female mammal follows a different time course than in the male. All cells in the oogonial stages progress to the oocyte stage in the embryo. By 3 days after birth, in the mouse or human, all of the oocytes are in a resting phase and there is no cell division. Consequently, in the adult there are no stem (oogonial) cells, but there are three types of follicles: immature, nearly mature, and mature.

The threshold for permanent sterility in women is an acute absorbed dose in the range 2.5 to 6 Gy (250-600 rad) or a protracted dose rate over many years of more than 0.2 Gy/y (20 rad/y). Pronounced hormonal changes, comparable to those associated with the natural menopause, accompany radiation-induced sterilization in females.

## REVIEW OF BASIC GENETICS

The study of the inheritance of observable characteristics includes molecular as well as morphologic and behavioral traits. The chromosomes carry in code form all of the information that specifies a particular human with all of his or her individual characteristics. The **chromosomes** are long thread-like structures, the essential ingredient of which is DNA, itself a long complex molecule with a sugar-phosphate backbone. Attached to each sugar molecule is an organic base; these come in four varieties: thymine, adenine, guanine, and cytosine. This whole configuration is tightly coiled in a double helix, rather like a miniature spiral staircase, with chains of sugar molecules linked by phosphates forming the rails on either side, bridged at regular intervals by pairs of bases, which form the steps. The order, or sequence, of the bases contains the genetic information in code form.

A **gene** is a finite segment of DNA specified by an exact sequence of bases. Genes occur along chromosomes in linear order like beads on a string, and the position of a gene is referred to as its locus.

The human chromosome complement consists of 22 pairs of autosomes present in both sexes, plus a pair of sex chromosomes, the X and Y. Males have 22 pairs of autosomes plus an X and a Y. Females have 22 pairs of autosomes plus a pair of Xs. One chromosome of each pair is derived from each parent.

The human genome is composed of the DNA of chromosomes and, to a minute extent, mitochondria. The 46 chromosomes contain about  $6 \times 10^9$  base pairs of DNA, with each chromosomal arm including a single supercoiled molecule of DNA associated with chromosomal proteins. The total number of genes is in the range of 50,000 to 100,000 per haploid set of chromosomes. The average gene therefore would contain 30 to 60 kilobases of DNA if no DNA is unrelated to genes. The study of individual genes hardly has begun, but it is apparent that some genes

are smaller than this and at least one, whose mutation can cause Duchenne-type muscular dystrophy, has been reported to contain more than 103 kilobases of DNA. Because most protein products of genes are less than 300,000 d, the translated portions of genes are seldom larger than 10 kilobases, so a major part of the genome appears to be untranslated. Some of this DNA appears to be transcribed but not translated. Much of the untranslated DNA consists of introns that reside between translated exons. In addition, much of the DNA outside the exons is involved in gene function, through regulation and RNA polymerase attachment.

Not only does this genome recombine in each generation, but it also undergoes **mutation**, a term used here to denote all changes in chromosomes, their genes, and their DNA. Thus, alterations in chromosome number and structure would be included along with changes not visible microscopically. These latter changes include an array of changes in DNA, such as deletion, rearrangement, breakage in the sugar-phosphate backbone, and base alterations. Gene function can be disturbed not only by loss or modification of translated exons but also through alteration of nonexonic sites that regulate transcription and translation. Mutation occurs in both germ cells and somatic cells, although it is much less apparent in the latter, unless it occurs under conditions of clonal proliferation, as happens with cancer. On the other hand, many mutations in the germline are lethal during embryonic development.

In humans, every normal cell has 46 chromosomes, 23 derived from the mother and 23 from the father. Each of a pair of chromosomes normally has the same genes for given characteristics lined up in the same sequence. In this case the two chromosomes are said to be **homologous**. The pair of chromosomes that determine sex are XX in the female and XY in the male; in the case of the male, therefore, the two chromosomes of this pair are **heterologous**: They do not contain parallel genes. If the two members of a pair of genes

are alike, the person is said to be **homozygous** for that pair of genes; if they are different, the person is said to be **heterozygous**.

The fact that pairs of chromosomes contain corresponding sets of genes introduces the idea of dominant and recessive genes. A dominant gene, by definition, expresses itself if its corresponding gene is recessive, the recessive gene in this case being either ineffective or suppressed. A completely recessive gene is expressed only in persons in whom both the corresponding genes of a pair of chromosomes are recessive (*i.e.*, the person must be homozygous for the recessive gene) or if the recessive gene is on the X chromosome in a male. Eye color is the simplest example. The gene for blue eyes is recessive; that for brown eyes is dominant. A child will have blue eyes only if he or she receives the gene for blue eyes from both parents. If both or only one of the genes that determine eye color is for brown eyes, then the child will have brown eyes, because this gene is dominant. It should be pointed out that not all genes are completely dominant; some permit expression of the recessive counterpart to a varying extent, depending on the particular characteristics involved.

The Y sex chromosome in humans has genes that determine maleness but appears to have few other genes. The X chromosome, on the other hand, has many genes. If a mother carries a recessive mutant gene on the single X chromosome that she donates to her son, there is no matching gene from the father, and consequently the recessive gene is expressed. If the offspring is a daughter, there may well be a dominant gene on the X chromosome supplied by the father, which would suppress the expression of the recessive mutant. The daughter, however, could transmit the mutant gene to her sons, in whom it would be expressed. Characteristics that result from recessive genes on the X chromosome, so that they are expressed almost exclusively in male children, are said to be sex-linked. The most common examples are color blindness and hemophilia.

An elementary discussion of genetics, such as that presented here, may give the impression that each characteristic of a person is determined by a single pair of genes. On the contrary, this is the exception rather than the rule, because most characteristics are the result of an interplay in the expression of many genes.

## MUTATIONS

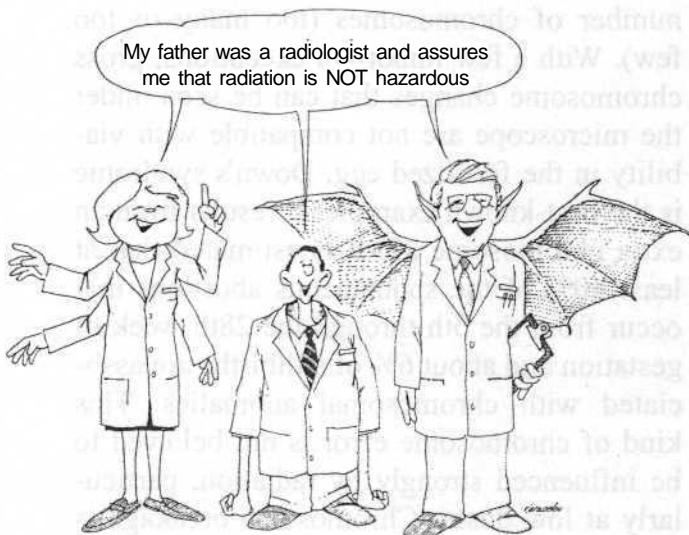
Exposure of a population to radiation can cause adverse health effects in the descendants as a consequence of mutations induced in the germ cells. Genetic diseases refer to those mutations occurring in the germ cells of parents and transmitted to progeny; that is, they are **hereditary diseases**, in contrast to most cancers, which result from mutations in somatic cells. Because the human genome includes between 50,000 and 100,000 genes, the potential number of mutations, and thus genetic diseases, is staggering.

It is a commonly held view that radiation produces bizarre mutants and monsters, as illustrated in Figure 11.1. This view is absolutely false. Radiation does not result in genetic effects that are new or unique but rather increases the frequencies of the same mutations that already occur spontaneously or naturally in that species.

Genetic diseases are classified into three principal categories: **mendelian**, **chromosomal**, and **multifactorial** (Table 11.1).

### Mendelian

Mendelian diseases are those that are caused by mutations in single genes located on either the autosomes or the sex chromosomes and that show simple predictable patterns of inheritance. The mutation may be a change in the structure of DNA, which may involve either the base composition, the sequence, or both. An alteration so small that it involves the substitution, gain, or loss of a single base can be the cause of significant inheritable changes. A striking example is sickle-



**Figure 11.1.** It is a commonly held view that radiation produces bizarre mutations or monsters that may be recognized readily. This is not true. Radiation increases the incidence of the same mutations that occur spontaneously in a given population. The study of radiation genetics is difficult, because the mutations produced by the radiation must be identified on a statistical basis in the presence of a high natural incidence of the same mutations.

cell anemia, which results from the substitution of only one base.

These diseases are subdivided into autosomal dominant, autosomal recessive, and X-linked conditions, depending on which chromosome the mutant genes are located on and the pattern of transmission. In the case of dominant diseases, one mutant gene received from either one of the parents is sufficient to cause disease, although the copy of the gene from the other parent is normal.

A **dominant** gene mutation is expressed in the first generation after its occurrence. More than 700 such conditions have been identified with certainty, and an additional 700 or more

are less well established. Some examples are polydactyly, achondroplasia, and Huntington's chorea.

By contrast, recessive mutations, unless sex-linked, require that the gene be present in duplicate to produce the trait, which means that the mutant gene must be inherited from each parent; consequently, many generations may pass before it is expressed. If one copy of the gene is mutant and the other is normal, the individual is not affected. More than 500 recessive diseases are known, and another 600 are suspected. Some examples are sickle-cell anemia, cystic fibrosis, and Tay-Sachs disease.

**TABLE 11.1. Heritable Effects of Radiation**

Heritable Effect	Example
Gene mutations <sup>a</sup>	
Single dominant 736 (753)	Polydactyly, Huntington's chorea
Recessive 521 (596)	Sickle-cell anemia, Tay-Sachs disease, cystic fibrosis, retinoblastoma
Sex-linked 80 (60)	Color blindness, hemophilia
Chromosomal changes	
• Too many or too few	Down's syndrome (extra chromosome 21), mostly embryonic death
Chromosome aberrations, physical abnormalities	Embryonic death or mental retardation
Robertsonian translocation	
Multifactorial!	
Congenital abnormalities present at birth	Neural tube defects, cleft lip, cleft palate
Chronic diseases of adult onset	Diabetes, essential hypertension, coronary heart disease

<sup>a</sup>The numbers following types of gene mutations refer to the number of human diseases known to be caused by such a mutation. The numbers in parentheses refer to additional possible diseases.

**X-linked** recessive diseases are caused by mutations in genes located on the X-chromosomes; compared with the X, the Y chromosome contains far fewer genes. Because males have only one X chromosome, all males having a mutation in the X chromosome show the effect of mutation; like dominant mutations, they are expressed soon after mutation occurs. Females, who have two X chromosomes, need two mutant genes to show the effect of an X-linked recessive mutation. The best known examples of **s&x-linked** disorders are hemophilia, color blindness, and a severe form of muscular dystrophy, but altogether there are more than 80 well-established and another 60 probable conditions of this sort.

In the case of mendelian diseases, about 67% are caused predominantly by point mutations (base-pair changes **in** the DNA), 22% by both point mutations and DNA deletions within genes (*i.e.*, intragenic), and 13% by intragenic deletions and large multilocus deletions. In some genes, the mutational sites of mutations are distributed throughout the gene; in a large proportion, however, these are nonrandomly distributed—that is, restricted to specific sites along the gene (specificities). Likewise, the break points of deletions are also nonrandomly distributed, showing specificities.

Some dominant and some recessive mutant genes cause traits that are regarded by society as normal or acceptable, such as different eye colors or blood groups. The majority, however, cause diseases ranging from mild to severe in their impact on the person.

The three types of Mendelian diseases are summarized as follows:

Autosomal dominant: due to mutations in a single gene on one chromosome

Autosomal recessive: defective copy of same gene from each parent

Sex linked: males have one X chromosome—one mutation can cause disease; females **have** two Xs—two mutant genes needed.

### Chromosomal Aberrations

Chromosomal diseases are caused by gross abnormalities either in the structure or in the

number of chromosomes (too many or too few). With a few important exceptions, gross chromosome changes that can be seen under the microscope are not compatible with viability in the fertilized egg. Down's syndrome is the best-known example: It results from an extra chromosome 23. It is estimated that at least 40% of the spontaneous abortions that occur from the 5th through the 28th week of gestation and about 6% of stillbirths are associated with chromosomal anomalies. This kind of chromosome error is not believed to be influenced strongly by radiation, particularly at low doses. Chromosome breakage is less frequent than aberrations among spontaneous instances of severe human anomalies, but radiation is much more effective at breaking chromosomes than in causing errors in chromosome distribution. Chromosomes that are broken may rejoin **in** various ways (Chapter 2). A translocation, for example, involves the reciprocal exchange of parts between two or more chromosomes and is not necessarily harmful as long as both rearranged chromosomes are present and contain the full gene complement. Children of a person with a translocation often receive only one of the rearranged chromosomes, and their cells are therefore genetically unbalanced. The nature and extent of the abnormality varies enormously, and the harm to the person ranges from rather mild to very severe. Chromosome imbalance, if it does not cause the death of the embryo, typically leads to physical abnormalities, usually accompanied by mental deficiency.

Robertsonian translocations are the most common type found in normal humans. These are fusions of two chromosomes, each having a spindle attachment at the end of the chromosome, to produce a single chromosome with the spindle attachment in the center. The children of a person with this type of translocation are usually normal, because they inherit either the translocated pair or a pair of normal chromosomes. Radiation does not appear to be a major cause of Robertsonian translocations but rather tends to induce those of the reciprocal-exchange type.

### Multifactorial Diseases

The term "multifactorial" is a general designation assigned to a disease known to have a genetic component but whose transmission patterns cannot be described as simple mendelian. The common congenital abnormalities that are present at birth (*e.g.*, neural tube defects, cleft lip with or without cleft palate) and many chronic diseases of adult onset, such as diabetes, essential hypertension, and coronary heart disease, are examples of multifactorial diseases. These diseases result from a number of causes, both genetic and environmental, the nature of which can vary among individuals, families, and populations. For these diseases, there is no simple relationship between mutation and disease, but the fact that genetic factors are involved is evident from observations of familial clustering; that is, these diseases run in families, but the recurrence risk to first-degree relatives is in the range between 5 and 10%, depending on the multifactorial disease, but never close to 50 or 25% characteristic of mendelian disease. The potential role of some of the environmental factors has been delineated for only a few of these diseases; for example, excess caloric intake rich in saturated fat is an environmental risk factor for coronary heart disease, environmental allergens for asthma. Mendelian and chromosomal diseases are rare and account for only a very small proportion of genetic diseases in the population; the major load is from multifactorial diseases.

Characteristics of multifactorial diseases include the following:

- Known to have a genetic component
- Transmission pattern not simple mendelian
- Congenital abnormalities: cleft lip with or without cleft palate; neural tube defects
- Adult-onset: diabetes, essential hypertension, coronary heart disease
- Interaction with environmental factors

### RADIATION-PRODUCED HEREDITARY EFFECTS

The fact that mutants produced by man-made radiations cannot be recognized or iden-

tified as different, compared with the natural spontaneous types, makes their study particularly difficult. Sample sizes must be large to detect a small increase caused by radiation.

Few human data are available on the hereditary effects of radiation, except the observations of hereditary consequences in the children of Japanese survivors of the atomic-bomb attacks on Hiroshima and Nagasaki. Consequently, the estimation of hereditary risks in the human must be based almost entirely on animal data.

### RELATIVE VERSUS DIRECT (OR ABSOLUTE) MUTATION RISK

There are essentially two ways to estimate the hereditary risks of radiation. The first is to compare radiation-induced mutations with those that occur spontaneously and to express the results in terms of the doubling dose—that is, the amount of radiation required to produce as many mutations as occur spontaneously in a generation. It is obtained by dividing the average spontaneous mutation rate at a set of genes by the average rate of induction by radiation at the same set of genes. The use of the concept of the doubling dose from animal experiments to estimate the risk of hereditary effects is based on the assumption that "if nature can do it, radiation can do it, too." This is the **relative mutation risk**.

The alternative approach is to ignore the natural or spontaneous rate and simply to quote the incidence of disorders resulting from mutations in the first generation. This is the **direct or absolute mutation risk**. The vast bulk of the useful data involves relative mutation risks, and this is discussed in some detail elsewhere in this chapter.

### RELATIVE MUTATION RISK ASSESSED IN THE MEGAMOUSE PROJECT

The husband-and-wife team of Russell and Russell, working at Oak Ridge National Laboratory, mounted an experiment of heroic proportions to determine specific locus mutation

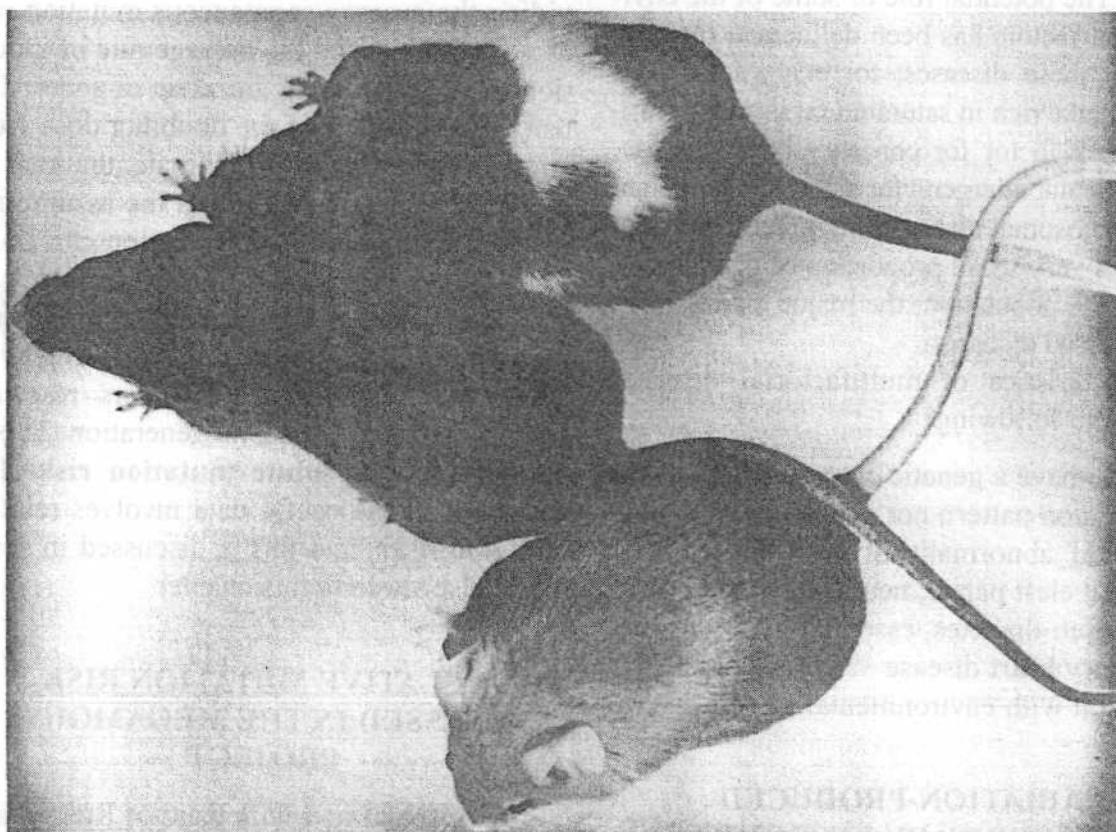
rates in the mouse under a variety of irradiation conditions. This experiment often is referred to as the "megamouse project" because of the enormous number of animals involved. Before the study ended, 7 million mice had been used.

An inbred mouse strain was chosen in which seven specific-locus mutations occur, six of which involve a change of coat color, and one expressed as a stunted ear. Figure 11.2 shows three coat-color variations: a piebald-, a light honey, and a darker brown. These mutations occur spontaneously, and their incidence is increased by radiation.

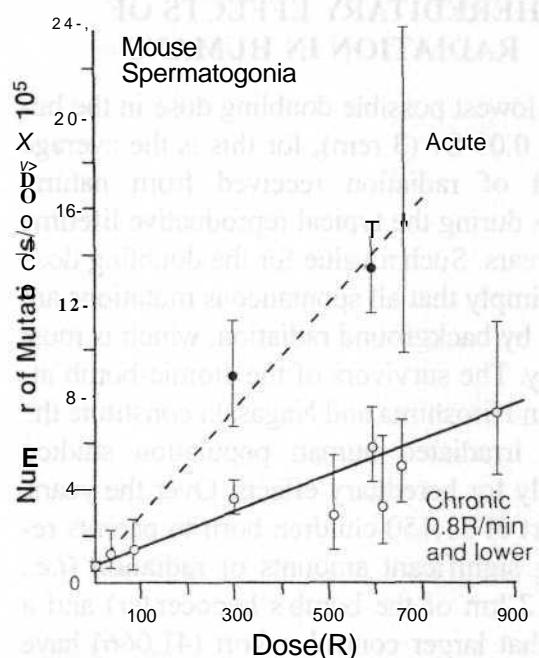
These extensive studies included the irradiation of both male and female mice with a range of doses, dose rates, and fractionation patterns. Of particular interest was a comparison of mutation rates induced by radiation in different stages of spermatogenesis. The results obtained are exceedingly complex; only

the briefest summary is presented in this chapter. Five major conclusions are pertinent to the radiologist:

1. The radiosensitivity of different mutations varies by a significant factor of about 35, so that it is only possible to speak in terms of average mutation rates.
2. In the mouse there is a substantial dose-rate effect, so that spreading the radiation dose over a period of time results in fewer mutations for a given dose than in an acute exposure. This is in complete contrast to the data on *Drosophila*. In the male there is a big dose-rate effect, between 90 and 0.8 R/mm (about 90 and 0.8 cGy/min), which the Russells attribute to a repair process. The data are shown in Figure 11.3.
3. The male is much more radiosensitive than the female. This is particularly true



**Figure 11.2.** In the megamouse project, seven specific-locus mutations were used to study radiation-produced genetic effects. This picture shows three of the mutations, which involve changes of coat color. (Courtesy of Dr. William L. Russell, Oak Ridge National Laboratory.)



**Figure 11.3.** Mutations in mice as a function of dose, delivered at high and low dose rates. (Courtesy of Dr. William L. Russell, Oak Ridge National Laboratory.)

at a low dose rate, at which it is doubtful if there is any increase in mutations in the female, even after a dose of several gray (several hundred rad). The difference between the sexes is so pronounced that, for practical purposes, at a low dose rate almost all of the radiation-induced genetic burden in a population is carried by the males.

4. The genetic consequences of a given dose can be reduced greatly if a time interval is allowed between irradiation and conception. This was first noticed in the male and a correlation found to exist with the stage of spermatogenesis at which the radiation was delivered. If animals were irradiated and used immediately for mating in genetic experiments, so that the sperm used for fertilization had been irradiated in a mature state, then a relatively large number of mutations were produced. In contrast, if animals were irradiated and mating delayed for a number of weeks, so that the sperm used for fertilization had been irradiated in a primitive state, then

fewer mutations were produced. Consequently, it is inferred that this decrease in mutation rate with time after irradiation is the consequence of some repair process. Whatever the explanation, it is an important empiric observation that the genetic consequences of a given dose of radiation can be reduced if a time interval is allowed between irradiation and conception. This information already is used in genetic counseling. In the mouse, a time interval between irradiation and conception of 2 months in the male and rather longer in the female is sufficient to produce a maximum reduction in the effect of radiation. Although data are not available for humans, by analogy a period of 6 months usually is recommended. Consequently, if persons are exposed to significant doses of radiation, either accidentally or as a result of their occupation, it is recommended that 6 months be allowed to elapse between the exposure to the radiation and a planned conception, to minimize the genetic consequence. This would be good advice to a person accidentally exposed to, say 0.1 Gy (10 rad), to young patients with Hodgkin's disease receiving radiotherapy, or even to patients subjected to diagnostic x-ray procedures involving the lumbar spine or the lower gastrointestinal tract, in which a large exposure is used and the gonads must be included within the radiation field.

5. The estimate of the doubling dose favored by the latest reports of the Committee on the Biological Effects of Ionizing Radiation (BEIR V) and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR 88) is 1 Gy (100 rad) based on low dose-rate exposure. This is a calculated rather than a measured quantity, based on the measured mutation rate per locus in the mouse, adjusted for the estimated comparable number of loci in the human. It also allows for the fact that the human usually is exposed at low dose-rate whereas the mouse data reflect acute exposures. It involves a number of

uncertainties. Earlier reports quoted a range of doses, rather than a single dose, including 0.2 to 2 Gy (20-200 rad) and 0.5 to 2.5 Gy (50-250 rad).

The BEIR and UNSCEAR Committees have depended almost entirely on the specific locus data from the mouse, because it is by far the biggest study of its kind. In recent years, however, the policy of basing hereditary effects in the human on mouse data has been called into question. There are several reasons for this.

1. The mouse doubling-dose data all come from male mice. Although the first few litters posttreatment of irradiated females showed genetic damage, later litters did not. This creates a dilemma for risk setting. Are human females like mice in this respect? To be conservative in extrapolating to the human situation, the mouse male-derived risks have been applied to both sexes, but this could lead to an uncertainty in the doubling dose by a factor of 2.
2. Most of the genes used in radiation mutation studies (involving for example coat-color changes) are either nonessential for viability or happen to be located in regions of the genome that can be deleted without loss of viability. For this reason, they are not representative of spontaneous mutations.
3. The mouse data are based on recessive mutations, which then are used to estimate the risk of dominant and X-linked diseases. If mechanistic and specificity differences between spontaneous and induced mutations are taken into account, the predicted risks are lower.
4. Cluster mutations and mosaic mutations were excluded from the calculation of the doubling dose.

As a consequence of these considerations, hereditary effects of radiation, already small, are likely to be even smaller in future editions of the BEIR and UNSCEAR reports.

## HEREDITARY EFFECTS OF RADIATION IN HUMANS

The lowest possible doubling dose in the human is 0.03 Sv (3 rem), for this is the average amount of radiation received from natural sources during the typical reproductive lifetime of 30 years. Such a value for the doubling dose would imply that all spontaneous mutations are caused by background radiation, which is most unlikely. The survivors of the atomic-bomb attacks on Hiroshima and Nagasaki constitute the largest irradiated human population studied carefully for hereditary effects. Over the years, a cohort of 31,150 children born to parents receiving significant amounts of radiation (*i.e.*, within 2 km of the bomb's hypocenter) and a somewhat larger control cohort (41,066) have been studied with respect to a variety of indicators: first, in the early years, for congenital defects, sex of child, physical development, and survival; then, in the middle years, for cytogenetic abnormality; and, more recently, for the occurrence of malignant disease and for electrophoretic or functional defects in a battery of some 30 serum proteins or erythrocyte enzymes. None of these indicators was related significantly to parental radiation exposure, but the net regression was slightly positive.

For these various measures of hereditary effects, the differences between the children of proximally and distally exposed survivors is in the direction expected if a hereditary effect did result from the radiation, but in fact none of the findings is statistically significant. Nevertheless, the differences between these groups of children were used to calculate doubling doses. It is argued that it is permissible to use those data to estimate doubling doses, even in the absence of a statistically significant effect, because the link between radiation exposure and hereditary anomalies is not in doubt, having been established unequivocally by animal data. In addition, of course, the chromosomal damage seen in the survivors suggests that this is the case.

Only three of these indicators lend themselves to an estimate of doubling dose, and the results are shown in Table 11.2, taken

TABLE 11.2. *Doubling Dose (Gametic) in the Offspring of Survivors of the Atomic-Bomb Attacks on Hiroshima and Nagasaki*

Genetic Indicator	Doubling Dose, Sv
Untoward pregnancy outcome	0.69
Childhood mortality	1.47
Sex chromosome aneuploidy	2.52
Simple average	1.56

Adapted from Schull WJ, Otake M, Neal JV: Genetic effects of the atomic bomb: A reappraisal. *Science* 213:1220-1227, 1981, with permission.

from a study in the early 1980s. The simple average of the three estimates is 1.56 Sv (156 rem). In a more recent review paper, Neel estimated the doubling dose for the human to be about 2 Sv; this is subject to considerable uncertainties, with a lower limit of 1 Sv and an upper limit that is indeterminate. This, of course, refers to an acute radiation dose, because it depends on the Japanese survivors.

This estimate for humans should be compared with the comparable figure of about 0.39 Gy (30 rad) for the doubling dose in the mouse for an acute exposure to low linear energy transfer radiation. (The figure of 1 Gy for the doubling dose quoted previously refers to a low dose rate.) The sparse human data do support the current impression that doubling doses derived from the mouse-specific locus experiments are too low.

The guiding principles for assessing hereditary risk in humans may be summed up as follows:

1. Most mutations, whether spontaneous or induced by radiation, are harmful.
2. Any dose of radiation, however small, entails some risk of hereditary effects; but radiation is a relatively weak mutagen.
3. The number of mutations produced is proportional to the dose, so that a linear extrapolation from high-dose data provides a valid estimate of low-dose effects.
4. Risk estimates are based on experiments with the mouse.

The conditions under which humans are exposed to radiation, whether as members of

the general public or in the course of their occupations, are such that mutation rates are low. Either the dose rate is very low in conditions of continuous exposure or, if high dose rates are involved, the dose per exposure is small. In either case, the mouse data indicate a low yield of mutations. On the rare occasions in which a large dose is absorbed in an acute exposure, as, for example, in a radiation accident, a significant proportion of the deleterious hereditary consequences can be avoided if conception is deferred. For a male 2 months is sufficient; the comparable period for females is not known with any certainty, but it is probably longer than for males. As suggested previously, a prudent and conservative approach might be to recommend to both males and females that planned conception should be delayed for at least 6 months after a significant irradiation exposure to minimize the hazard of hereditary anomalies.

## NUMERICAL VALUES OF HEREDITARY RISK

For more than a decade, both the BEIR and UNSCEAR committees have used a doubling dose of 1 Gy for hereditary effects in the human, based on the experimental data in the mouse. This figure does not include any allowance for a hereditary component to multifactorial disorders; both committees acknowledge that this may be an important effect of radiation but were unable to make a realistic estimate.

For a working population, the International Commission on Radiological Protection estimates the probability *per caput* for radiation-induced hereditary disorders to be  $0.6 \times 10^{-2}$  per sievert. This is based on the doubling dose of 1 Gy (100 rad), recommended by BEIR and UNSCEAR, plus an approximate allowance for multifactorial diseases. This risk, of course, is additional to that for cancer. As previously discussed, the doubling dose of 1 Gy is almost certainly too low, and future estimates of hereditary risks are likely to be revised downwards.

### SUMMARY OF PERTINENT CONCLUSIONS

- In the male, the threshold for temporary sterility is about 0.15 Gy (15 rad), but the effect is not apparent immediately. The threshold for permanent sterility is about 3.5 to 6 Gy (350-600 rad).
- In the female, the threshold for permanent sterility is 2.5 to 6 Gy (250-600 rad).
- The induction of sterility in males does not produce significant changes in hormone balance, libido, or physical capability but in the female leads to pronounced hormonal changes comparable to natural menopause.
- Exposure of a population can cause adverse health effects in the descendants as a consequence of mutations induced in germ cells.
- Hereditary diseases are classified into three principal categories: **mendelian, chromosomal, and multifactorial**.
- Radiation does not produce new, unique mutations but increases the incidence of the same mutations that occur spontaneously.
- Information on the hereditary effects of radiation comes almost entirely from animal experiments.
- Relative mutation rates have been measured in the megamouse project by observing specific locus mutations. This leads to an estimate of the doubling dose.
- The doubling dose is the dose required to double the spontaneous mutation incidence; put another way, it is the dose required to produce an incidence of mutations equal to the spontaneous rate. Based on the mouse data, the doubling dose for low dose rate exposure in humans is estimated to be 1 Gy (100 rad). For a number of reasons this is probably too low, and it does not include an allowance for multifactorial diseases.
- Not more than 1 to 6% of spontaneous mutations in humans may be ascribed to background radiation.
- Children of the atomic-bomb survivors have been studied for a number of indicators, including congenital defects, sex ratio, survival, cytogenetic abnormalities, and electrophoretic variants of blood proteins. The doubling dose is estimated to be about 2 Sv, with a lower limit of 1 Sv and an upper limit that is indeterminate, because the increase in mutations is not statistically significant.
- In terms of detriment, expressed in years of life lost or impaired, congenital anomalies (*i.e.*, resulting from effects on the developing embryo and fetus) are much more important than genetically transmitted disorders.
- For a working population, the International Commission on Radiological Protection estimates the probability *per caput* for severe radiation-induced hereditary disorders to be  $0.6 \times 10^{-2}$  per sievert. This is based on a doubling dose of 1 Gy, plus an approximate allowance for multifactorial diseases.
- Because the doubling dose of 1 Gy, based on specific locus mutations in mice, is probably too low, future risks of hereditary disorders, already small, are likely to be further reduced

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# 12

## Effects of Radiation on the Embryo and Fetus

HISTORICAL PERSPECTIVE  
OVERVIEW OF RADIATION EFFECTS ON  
THE EMBRYO AND FETUS  
DATA FROM RATS AND MICE  
EXPERIENCE IN HUMANS  
SURVIVORS OF THE A-BOMB ATTACKS ON  
HIROSHIMA AND NAGASAKI  
*IRRADIATED IN UTERO*  
EXPOSURE TO MEDICAL RADIATION

COMPARISON OF HUMAN AND ANIMAL  
DATA  
CANCER IN CHILDHOOD AFTER  
*IRRADIATION /A<sup>r</sup> UTERO*  
OCCUPATIONAL EXPOSURE OF WOMEN  
THE PREGNANT OR POTENTIALLY  
PREGNANT PATIENT  
SUMMARY OF PERTINENT CONCLUSIONS

### HISTORICAL PERSPECTIVE

In the early years of this century, case reports began to appear in the medical literature that described mental retardation in children with small head size, as well as other gross malformations, born to mothers who had inadvertently received pelvic radiotherapy during pregnancy. As early as 1929, Goldstein and Murphy reviewed 38 such cases. Interestingly enough, they concluded that large doses were needed to produce such effects and did not consider diagnostic pelvic irradiation of the mother to be a hazard.

We now have a great deal of information concerning the effects of radiation on the developing embryo and fetus from both animal experiments and the human experience.

### OVERVIEW OF RADIATION EFFECTS ON THE EMBRYO AND FETUS

Among the somatic effects of radiation other than cancer, developmental effects on the unborn child are of greatest concern. The classic effects are listed here:

1. *Lethal effects*, induced by radiation before or immediately after implantation of the embryo into the uterine wall or induced after increasingly higher doses during all stages of intrauterine development, to be expressed either before birth (prenatal death) or at about the time of birth (neonatal death)
2. *Malformations*, characteristic of the period of major organogenesis, in which the main body structures are formed, and especially of the most active phase of cell multiplication in the relevant structures
3. *Growth disturbances without malformations*, induced at all stages of development but particularly in the latter part of pregnancy

The principal factors of importance are the *dose* and the *stage of gestation at which it is delivered*. *Dose rate* is also of significance, because many pathologic effects on the embryo are reduced significantly by reducing the dose rate.

It should be recognized that congenital anomalies arise in all animal species, even in

the absence of any radiation beyond that received from natural sources. The incidence depends to a large extent on the time at which the anomalies are scored. The incidence of malformed infants at birth is about 6%, averaged for the human species. Some malformations disappear after birth, but more become evident later than are not scored at birth. The global incidence roughly doubles to 12% if grown children rather than infants are examined. Any assessment of the effectiveness of radiation in inducing damage *in utero* must be viewed against this natural level of inborn defects and its variable expression.

### DATA FROM RATS AND MICE

Most experimental data on the effect of radiation in the developing embryo or fetus have been obtained with the mouse or rat, animals that reproduce in quantity with relatively short gestation periods. Russell and Russell divided the total developmental period *in utero* into three stages: (1) **preimplantation**, which extends from fertilization to the time at which the embryo attaches to the wall of the uterus; (2)

**organogenesis**, the period during which the major organs are developed; and (3) **the fetal stage**, during which growth of the structures already formed takes place. There is a very large variability in the relative duration of these periods among animal species, as well as in the total duration of intrauterine life. Also, at any given stage of development, the state of differentiation or maturation of any one structure, with respect to all the others, varies considerably in different species.

In the mouse, preimplantation corresponds to days 0 through 5; organogenesis to days 5 through 13; and the fetal period from day 13 through full term, which is about 20 days. The effect of 2 Gy (200 rad) delivered at various times after conception is illustrated in Figure 12.1. The lower scale contains Rugh's estimates of the equivalent ages for human embryos, based solely on comparable stages of organ development. It is a nonlinear match, because preimplantation, organogenesis, and the fetal period in the mouse are about equal in length, whereas the fetal period in the human is proportionately much longer.

Figure 12.2 is taken from the work of Brent and Ghosson, who have performed an exten-

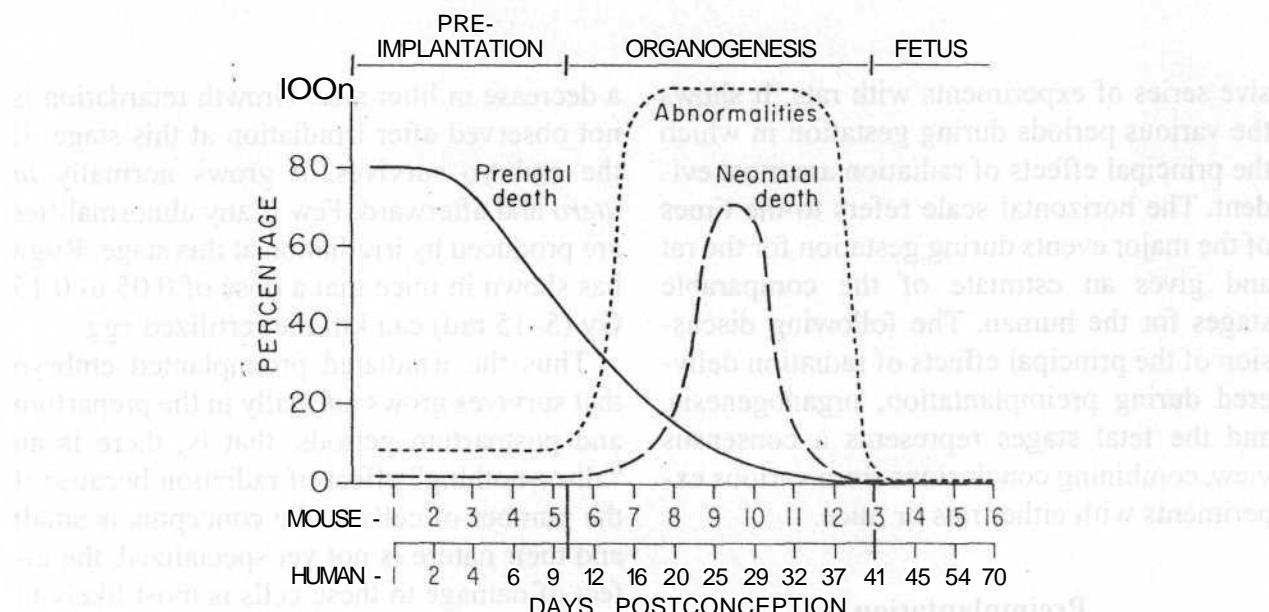
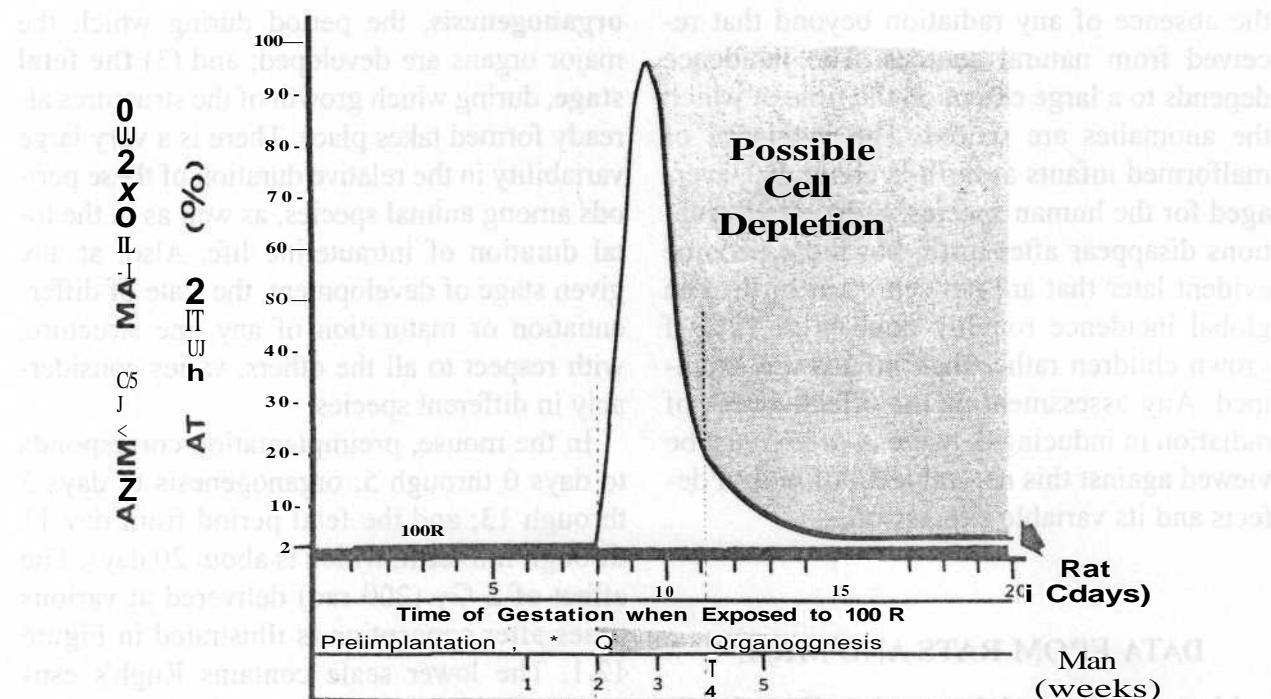


Figure 12.1. Incidence of abnormalities and of prenatal and neonatal death in mice given a dose of 200 R at various times after fertilization. The lower scale consists of Rugh's estimates of the equivalent stages for the human embryo. (Data from Russell LB, Russell WL: An analysis of the changing radiation response of the developing mouse embryo. J Cell Physiol 43[suppl 1]: 1030-149, 1954.)



**Figure 12.2.** Relative incidence of congenital malformations in the rat after an x-ray exposure of 100 R, delivered at various stages during gestation. The control incidence in this species is about 2%, indicated by the arrow on the right. The incidence of malformation after irradiation before the ninth day is not detectably different from controls. A large incidence, approaching 100%, occurs if the radiation is delivered during early organogenesis, corresponding to the third and fourth week of a human pregnancy. The number of malformations produced falls off rapidly as organogenesis diminishes, though some organogenesis of the central nervous system continues to term. During the fetal stage, a dose of 100 R causes an irreversible loss of cells that is expressed as growth retardation persisting to adulthood. The asterisk shows the stage in implantation in which radiation causes growth retardation that is expressed as a decrease in weight at term. (From Brent RL, Ghosson RO: Radiation exposure in pregnancy. *Curr Probl Radiol* 2:1-48, 1972, with permission.)

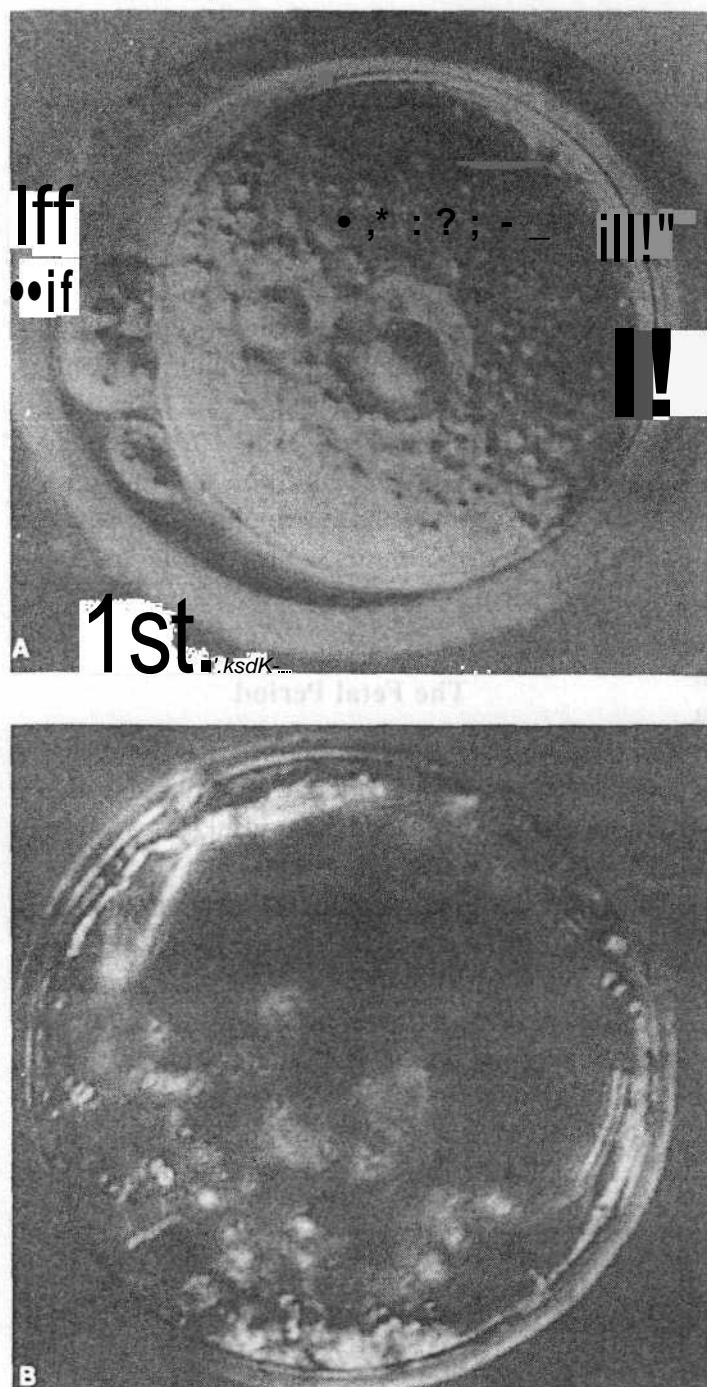
sive series of experiments with rats. It shows the various periods during gestation in which the principal effects of radiation are most evident. The horizontal scale refers to the times of the major events during gestation for the rat and gives an estimate of the comparable stages for the human. The following discussion of the principal effects of radiation delivered during preimplantation, organogenesis, and the fetal stages represents a consensus view, combining conclusions from various experiments with either rats or mice.

#### Preimplantation

Preimplantation is the most sensitive stage to the lethal effects of radiation. This high incidence of prenatal death may be expressed in

a decrease in litter size. Growth retardation is not observed after irradiation at this stage; if the embryo survives, it grows normally *in utero* and afterward. Few if any abnormalities are produced by irradiation at this stage. Rugh has shown in mice that a dose of 0.05 to 0.15 Gy (5-15 rad) can kill the fertilized egg.

Thus the irradiated preimplanted embryo that survives grows normally in the prepartum and postpartum periods, that is, there is an "all-or-nothing" effect of radiation because if the number of cells in the conceptus is small and their nature is not yet specialized, the effect of damage to these cells is most likely to take the form of a failure to implant or an undetected death of the conceptus. This is illustrated in the remarkable pictures produced by Pedersen and reproduced in Figure 12.3. If



**Figure 12.3.** During preimplantation, the embryo consists of a limited number of cells. A: Newly fertilized mouse egg. B: By the third day, the mouse embryo consists of only 16 cells. About 5 days after conception in the mouse, which corresponds to 9 or 10 days in the human, the embryo becomes embedded in the wall of the uterus, and at about this time cells begin to differentiate to form specific tissues and organs. (Courtesy of Dr. Pedersen, University of California at San Francisco.)

too many cells are killed by irradiation, the embryo dies and is resorbed. If only a few cells are killed, one or two cell divisions can make good the damage.

#### Organogenesis

During organogenesis, the principal effect of radiation in small rodents is the production of a variety of congenital anomalies of a

structural nature. As seen from Figure 12.1, a dose of about 2 Gy (200 rad) to the mouse embryo during the period of maximum sensitivity can result in a 100% incidence of malformations at birth. A similar result is seen for rats exposed to about 1 Gy (100 rad) in Figure 12.2. During organogenesis, most of the embryonic cells are in their blastula, or differentiating, stage and are particularly sensitive. This is the period in the human in which the

tranquilizer thalidomide produced such disastrous effects (at about 35 days after conception) and is also the time of maximum risk of deleterious effects from the rubella virus.

Examples of gross anomalies resulting from irradiation during the period of organogenesis are shown in Figures 12.4 and 12.5. It is characteristic of mice and rats that a wide variety of structural malformations are seen. The production of a specific defect is associated with a definite time during this period of organogenesis, usually the time of the first morphologic evidence of differentiation in the organ or portion of the organ involved.

Embryos exposed during early organogenesis also exhibit the greatest intrauterine growth retardation. This is expressed as a weight reduction at term and is a phenomenon resulting from cell depletion. Animals show a remarkable ability to recover from the growth retardation produced by irradiation during organogenesis, and although they may be smaller than usual at birth they may achieve a normal weight as adults. There is an association between growth retardation and teratogenesis: Irradiated embryos that show major congenital anomalies also suffer an overall reduction of growth. In animals a dose of about 1 Gy (100 rad) produces growth retardation if

delivered at any stage of gestation (except during preimplantation); 0.25 Gy (25 rad) does not produce an observable effect even at the most sensitive stage.

If death occurs as a result of irradiation in organogenesis, it is likely to be neonatal death—occurring at or about the time of birth. The transition from prenatal death from irradiation during preimplantation to neonatal death resulting from irradiation in organogenesis is very clear from Figure 12.1. In this case, neonatal deaths peak at 70% for mice receiving about 2 Gy (200 rad) on the 10th day. The deaths probably occur because some grossly abnormal fetuses are unable to develop to term.

#### The Fetal Period

The remainder of pregnancy, the fetal period, extends from about day 14 onward in the mouse; this corresponds to 6 weeks onward in the human. A variety of effects have been documented in the experimental animal after irradiation during the fetal stages, including effects on the hematopoietic system, liver, and kidney, all occurring, however, after fairly high radiation doses. The effects on the developing gonads have been documented particu-

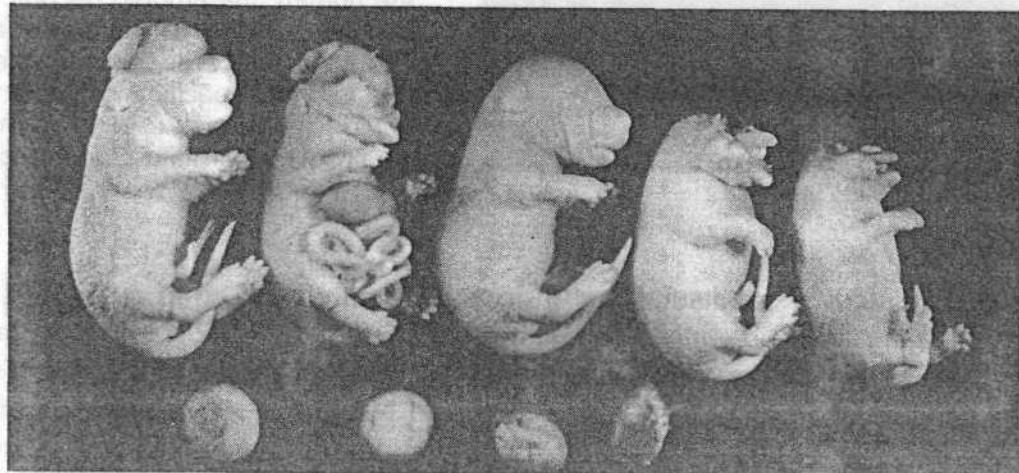
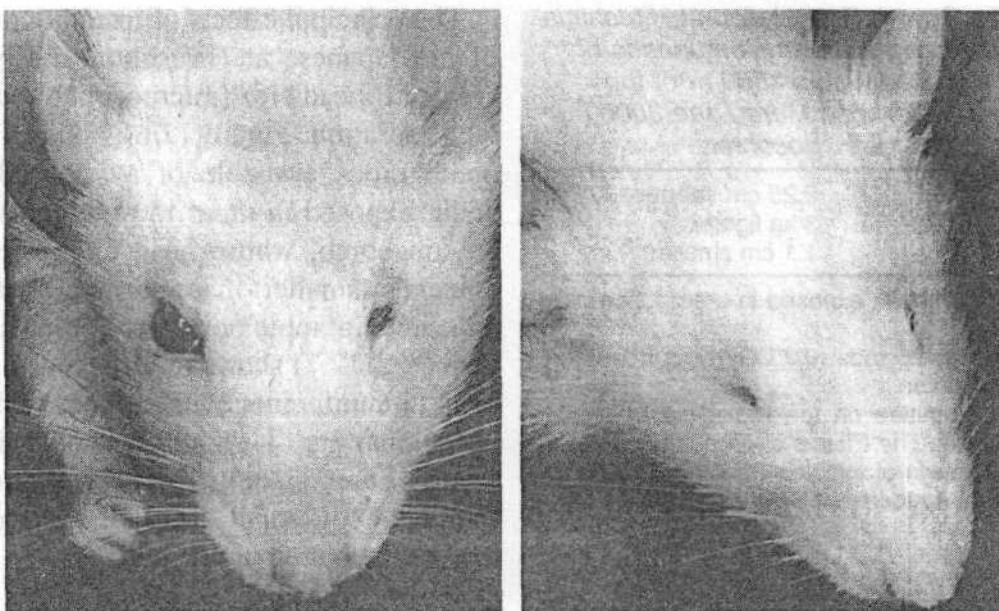


Figure 12.4. Litter from a female mouse irradiated with x-rays and sacrificed at 19 days. At least four different anomalies are demonstrated in this litter. There are four resorbed fetuses (below) and five fetuses alive. From left to right, the first shows exencephaly; the second, exencephaly and evisceration; the third is apparently normal; and the remaining two are anencephalics with stunting. (Photograph by Dr. Roberts Rugh.)



**Figure 12.5.** Two rats from the same litter exposed to a dose of 100 R of x-rays 9.5 days after conception. The rat on the left has a normal right eye and microphthalmus of the left. The rat on the right shows anophthalmia of both eyes. (From Rugh R, Caveness WF, Duhamel L, Schwarz GS: Structural and functional [electroencephalographic] changes in the post-natal mammalian brain resulting from x-irradiation of the embryo. *Mil Med* 128:392, 1959, with permission.)

larly well, both morphologically and functionally. There appears at present to be little correspondence between the cellular and functional damage as a function of dose, but doses of a few tenths of a gray as a minimum are necessary to produce fertility changes in various animal species.

Much higher doses of radiation are required to cause lethality during this period than at earlier stages of development, although the irradiated early fetus exhibits the largest degree of permanent growth retardation, in contrast to the embryo in early organogenesis, which exhibits the most temporary growth retardation, which is evident at term but from which the animal is able to recover later.

#### EXPERIENCE IN HUMANS

Information on the irradiation of human concepti comes from two major sources: medical exposures (particularly therapeutic irradiations), especially during the early part of the century, when hazards were not yet fully appreciated; and studies of atomic-bomb survivors in Japan.

The list of human abnormalities reported after *in utero* irradiation is long. Most commonly reported are microcephaly (sometimes combined with mental retardation), some other central nervous system defects, and growth retardation.

#### SURVIVORS OF THE ATOMIC-BOMB ATTACKS ON HIROSHIMA AND NAGASAKI IRRADIATED IN UTERO

The growth to maturity of children exposed *in utero* at Hiroshima and Nagasaki has been studied carefully. There are difficulties associated with the dosimetry, but the conclusions have far-reaching implications.

Data on those children exposed *in utero* in Hiroshima and Nagasaki show too few persons who were younger than 4 weeks of gestational age at the time the bomb was dropped. This deficiency presumably results from increased fetal loss or infant mortality rate. This stage of development is so early that damage to a single cell, or a group of cells, is likely to impair the function of all the progeny and lead to death of the embryo. In accord

**TABLE 12.1.** Growth Retardation at Hiroshima From In Utero Irradiation<sup>a</sup> Comparison of Those Exposed Within 1500 m of the Hypocenter With Those More Than 3000 m From the Hypocenter

Height	2.25 cm shorter
Weight	3 kg lighter
Head diameter	1.1 cm smaller

<sup>a</sup>80% of 1613 children exposed in utero followed to age 17 years.

"Average kerma, 25 rads (0.25 Gy), but doses are subject to modification.

Data from Committee on the Biological Effects of Ionizing Radiations: The Effects on Populations of Exposure to Low Levels of Ionizing Radiation. Washington, DC, National Academy of Sciences, 1980.

with this reasoning is the observation that no birth defects were found as a result of irradiation before 15 days of gestational age. This is in accord with the experimental data for rats and mice in which exposure during preimplantation had an all-or-none effect: death of the embryo or normal development.

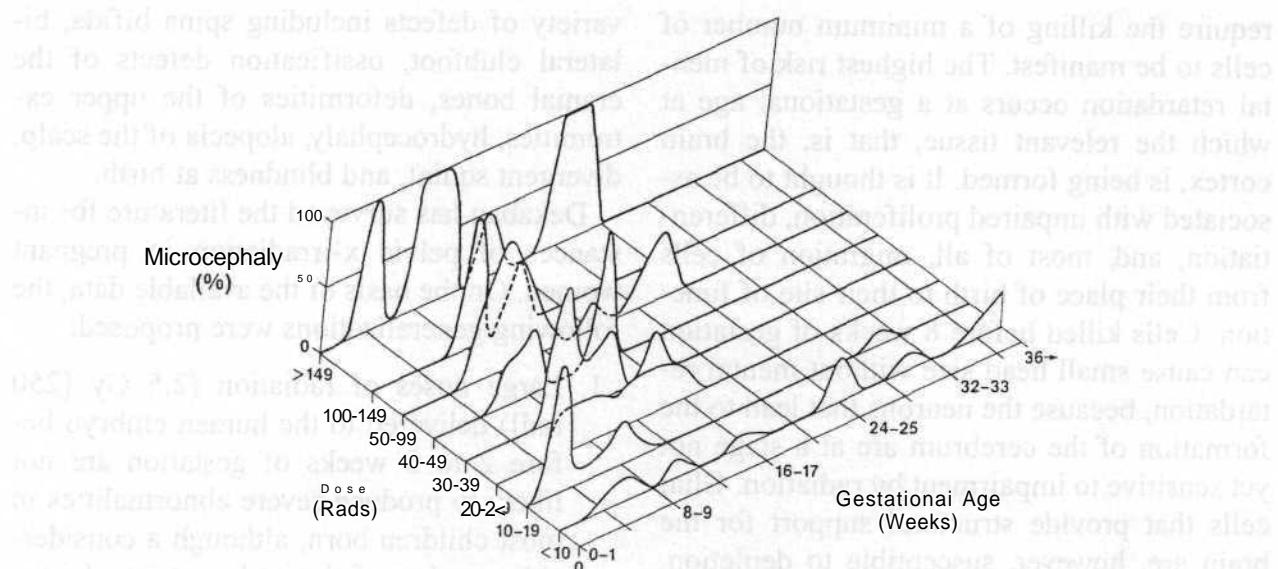
Exposure to radiation resulted in growth retardation (Table 12.1). Children exposed as embryos closer than 1,500 m to the hypocenter of the atomic explosion were shorter, weighed less, and had head diameters significantly smaller than children who were more than 3,000 m from the hypocenter and received negligibly small doses. It is of interest to note that there was no catch-up growth, because the smallness in head size was maintained into adulthood.

The principal effects of irradiation *in utero* of the Japanese at Hiroshima and Nagasaki are small head size (microcephaly) and mental retardation. Figure 12.6 is one of the few photographs available of a young Japanese adult, exposed *in utero* to radiation from the atomic bomb, whose head circumference is evidently smaller than normal. A three-dimensional graphic portrayal of the Hiroshima data (Fig. 12.7) shows the frequency of small head circumference with respect to dose and gestational age. It should be noted that each point is based on only a few observations. The frequency of small head circumference is most pronounced in the most heavily exposed group (1.5 Gy, *i.e.*, 150 rad or more), but that the effect is seen in those whose maternal exposure was 0.1 to 0.19 Gy (10-19 rad).

The prevalence of mental retardation in children exposed *in utero* to the atomic bombs in Hiroshima and Nagasaki also has been reevaluated in reference to gestational age and tissue dose in the fetus. The study involved about 1,600 children and confirmed that about 30 of them showed clinically severe mental retardation. A child was deemed to be mentally retarded if he or she was "unable to perform simple calculations, to make simple conversation, to care for himself or herself, or if he or she was completely unmanageable or has been institutionalized." Most of these children never were enrolled in public schools, but among the few who were, the highest IQ was 68.

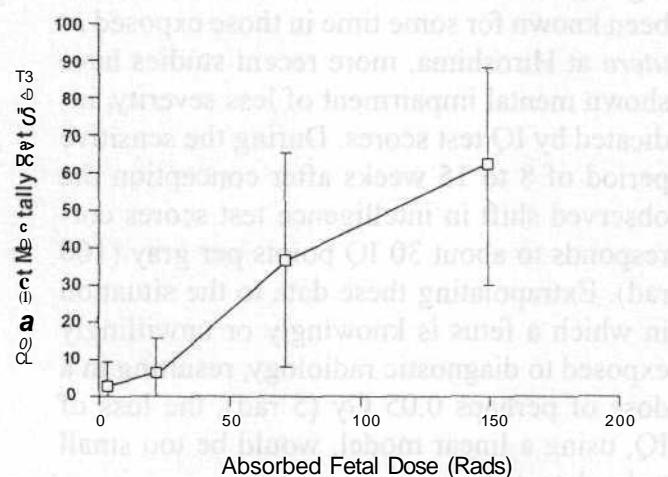


**Figure 12.6.** One of the few photographs available of a Japanese youth with reduced head circumference as a result of radiation exposure *in utero* from the atomic bomb. (From Committee for the Compilation of Materials on Damage Caused by the Atomic Bomb in Hiroshima and Nagasaki: Hiroshima and Nagasaki: The Physical, Medical and Social Effects of the Atomic Bombings. New York, Basic Books, 1981, with permission.)



**Figure 12.7.** Incidence of microcephaly as a function of dose and gestational age among children in Hiroshima exposed *in utero* to the atomic bomb. (Adapted from Miller RW, Mulvihill JJ: Small head size after atomic irradiation. In Sever JL, Brent RL [eds]: Teratogen Update: Environmentally Induced Birth Defect Risks, pp 141-143. New York, Alan R Liss, 1986, with permission.)

Of the 30 children judged to be severely mentally retarded, in five this was considered to be the consequence of causes other than radiation, including Down's syndrome, neonatal jaundice, encephalitis, or birth trauma; nevertheless, the remaining number represents an incidence far higher than normal. Severe mental retardation was not observed to be induced by radiation before 8 weeks after conception or after 25 weeks. The most sensitive period is 8 to 15 weeks after conception; for exposure during weeks 16 to 25, the risk is four times smaller. Figure 12.8 shows the relation between the incidence of mental retardation and absorbed dose for this most sensitive period. The relationship appears to be linear, and the data are consistent with a probability of occurrence of mental retardation of 40% at a dose of 1 Gy (100 rad). The possibility of a dose threshold cannot be excluded. By using the most recent dosimetry and discarding two cases of mental retardation for which *in utero* irradiation was unlikely to be the cause, the dose-response relationship is consistent with a threshold of 0.12 to 0.2 Gy (12-20 rad). A linear, nonthreshold response is unlikely in view of the presumed deterministic nature of mental retardation that would



**Figure 12.8.** The frequency of mental retardation as a function of dose among those exposed *in utero* to atomic-bomb radiation. The data are pooled from Hiroshima and Nagasaki for those exposed at 8 to 15 weeks gestational age. The vertical bars represent the 90% confidence intervals. There was no risk at 0 to 8 weeks after conception, and for exposure at later periods during gestation the excess is barely significant, even at the higher doses. (Adapted from Otake M, Schull WJ: *In utero* exposure to A-bomb radiation and mental retardation: A reassessment. Br J Radiol 57:409-414, 1984, with permission.)

require the killing of a minimum number of cells to be manifest. The highest risk of mental retardation occurs at a gestational age at which the relevant tissue, that is, the brain cortex, is being formed. It is thought to be associated with impaired proliferation, differentiation, and, most of all, migration of cells from their place of birth to their site of function. Cells killed before 8 weeks of gestation can cause small head size without mental retardation, because the neurons that lead to the formation of the cerebrum are at a stage not yet sensitive to impairment by radiation. Glial cells that provide structural support for the brain are, however, susceptible to depletion. Magnetic resonance images of individuals irradiated *in utero* at 3 to 15 weeks' gestation show evidence of massive impairment of cells to migrate from proliferative zones. Atypical distribution of gray matter often is seen in patients with mental retardation, but it is usually *unilateral*] that caused by radiation exposure is *bilateral*.

Although severe mental retardation requiring the children to be institutionalized has been known for some time in those exposed *in utero* at Hiroshima, more recent studies have shown mental impairment of less severity, indicated by IQ test scores. During the sensitive period of 8 to 15 weeks after conception the observed shift in intelligence test scores corresponds to about 30 IQ points per gray (100 rad). Extrapolating these data to the situation in which a fetus is knowingly or unwillingly exposed to diagnostic radiology, resulting in a dose of perhaps 0.05 Gy (5 rad), the loss of IQ, using a linear model, would be too small to be detected.

## EXPOSURE TO MEDICAL RADIATION

A relationship between microcephaly and x-irradiation during intrauterine life has been recognized since Murphy and Goldstein first focused attention on the subject in 1929. The numbers are small and the doses are not known with any certainty, though most were in the therapeutic range. Microcephaly was reported as well as mental retardation and a

variety of defects including spina bifida, bilateral clubfoot, ossification defects of the cranial bones, deformities of the upper extremities, hydrocephaly, alopecia of the scalp, divergent squint, and blindness at birth.

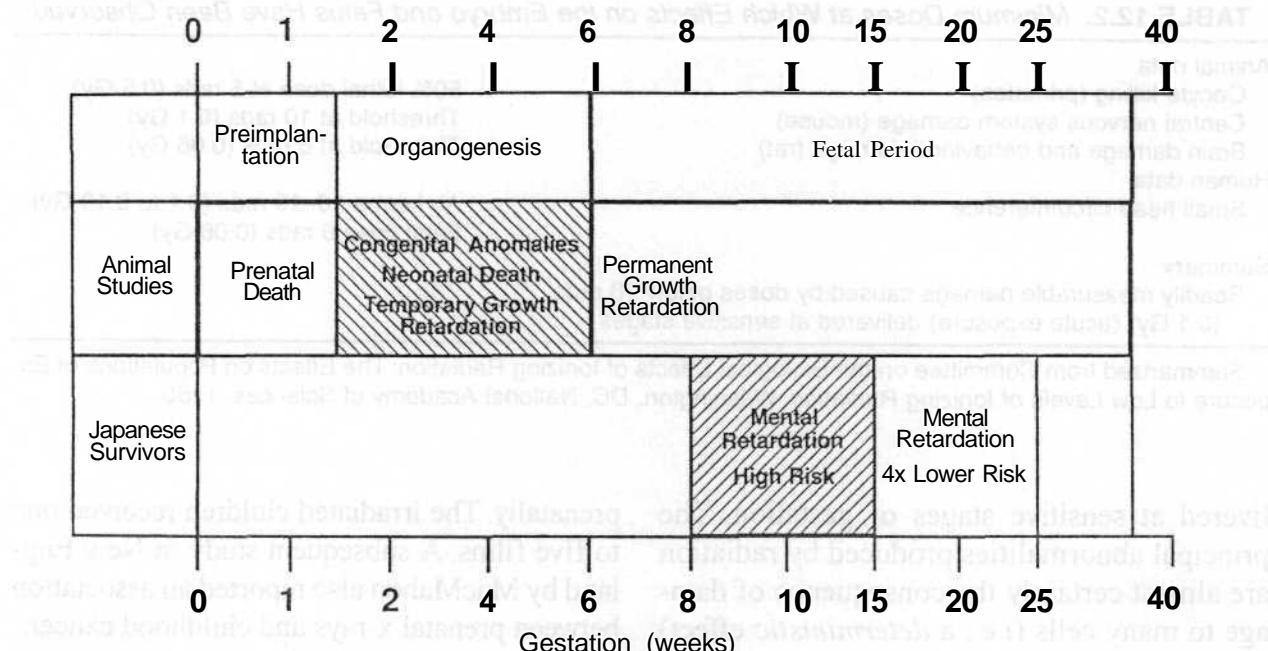
Dekaban has surveyed the literature for instances of pelvic x-irradiation in pregnant women. On the basis of the available data, the following generalizations were proposed:

1. Large doses of radiation (2.5 Gy [250 rad]) delivered to the human embryo before 2 to 3 weeks of gestation are not likely to produce severe abnormalities in most children born, although a considerable number of the embryos may be resorbed or aborted.
2. Irradiation between 4 and 11 weeks of gestation would lead to severe abnormalities of many organs in most children.
3. Irradiation between 11 and 16 weeks of gestation may produce a few eye, skeletal, and genital organ abnormalities; stunted growth, microcephaly, and mental retardation are frequently present.
4. Irradiation of the fetus between 16 and 20 weeks of gestation may lead to a mild degree of microcephaly, mental retardation, and stunting of growth.
5. Irradiation after 30 weeks of gestation is not likely to produce gross structural abnormalities leading to a serious handicap in early life but could cause functional disabilities.

## COMPARISON OF HUMAN AND ANIMAL DATA

Figure 12.9 is an attempt to summarize the data for the effects of radiation on the developing embryo and fetus, comparing and contrasting the information from animals and humans.

Exposure to radiation during preimplantation leads to a high incidence of embryonic death, but embryos that survive develop normally. This has been shown clearly in experiments with both rats and mice and is consistent with the data from Japan. In animals, irradiation during organogenesis leads to



**Figure 12.9.** Chart illustrating the similarities and differences between data from small laboratory animals, and data from the Japanese survivors of the atomic-bomb attacks. Both agree that irradiation early in gestation may result in the death of the embryo but that malformations do not occur. The animal data show a high incidence of a wide spectrum of malformations during organogenesis. The principal finding in the Japanese is microcephaly and mental retardation, which occurred most frequently following irradiation at 8 to 15 weeks of gestation and, to a lesser extent, at 15 to 25 weeks.

neonatal death, temporary growth retardation, and, above all, a wide range of malformations affecting many different limbs and organs. By contrast, the principal effect in the Japanese survivors of the atomic-bomb attacks is microcephaly with or without mental retardation, and this begins in the eighth week, that is, after the period classically described as organogenesis. The wide array of congenital malformations found in rats and mice irradiated in organogenesis was not reported in the Japanese survivors. Much has been made of this difference. On the one hand, it has been suggested that the gross structural deformities in Japan simply were not recorded in the chaos that followed the dropping of the atomic bombs. On the other hand, it is argued that humans differ from rats and mice in that the period of susceptibility to a wide array of congenital malformations (10-32 days of gestation) is short compared with the 8 weeks during which mental retardation can be induced and the 16 weeks during which irradiation can result in a reduced head diameter. Be-

cause the number of children involved is quite small, it might be expected that effects on the central nervous system, which is developing over a larger period of time, would dominate. The situation is different in laboratory animals: Their susceptibility to radiation-induced small head size is of similar duration to that for the induction of other deformities. The data from patients exposed to therapeutic doses of medical radiation show a range of congenital malformations that more closely mirrors the animal results, although the numbers are small and the doses high. To be on the safe side, it must be assumed that the entire period of gestation from about 10 days to 25 weeks is sensitive to the induction of malformations by radiation.

Table 12.2 summarizes the lowest doses at which effects on the embryo and fetus have been observed. This table summarizes the conclusions of the third report of the Committee on the Biological Effects of Ionizing Radiation. Readily measurable damage can be observed at doses below 0.1 Gy (10 rad) de-

TABLE 12.2. Minimum Doses at Which Effects on the Embryo and Fetus Have Been Observed

Animal data	
Oocyte killing (primates)	50% lethal dose at 5 rads (0.5 Gy)
Central nervous system damage (mouse)	Threshold at 10 rads (0.1 Gy)
Brain damage and behavioral damage (rat)	Threshold at 6 rads (0.06 Gy)
Human data	
Small head circumference	Air kerma 10-19 rads (0.1 to 0.19 Gy) Fetal dose 6 rads (0.06 Gy)
Summary	
Readily measurable damage caused by doses below 10 rads (0.1 Gy) (acute exposure) delivered at sensitive stages	

Summarized from Committee on the Biological Effects of Ionizing Radiation: The Effects on Populations of Exposure to Low Levels of Ionizing Radiation. Washington, DC, National Academy of Sciences, 1980.

livered at sensitive stages of gestation. The principal abnormalities produced by radiation are almost certainly the consequence of damage to many cells (*i.e.*, a *deterministic* effect) and therefore would be consistent with a threshold in dose. There is some indication of this in the data for mental retardation.

### CANCER IN CHILDHOOD AFTER IRRADIATION IN UTERO

The Oxford Survey of Childhood Cancers, published by Stewart and Kneale in the 1950s, suggested an association between the risk of cancer, principally leukemia, up to 15 years of age and exposure *in utero* to diagnostic x-rays. This was a retrospective case-controlled study and is summarized in Table 12.3. Of 7,649 children who died of leukemia or childhood cancers, 1,141 had been x-rayed *in utero*. Of an equal number of controls who did not develop childhood cancer, only 774 had been irradiated

prenatally. The irradiated children received one to five films. A subsequent study in New England by MacMahon also reported an association between prenatal x-rays and childhood cancer.

This subject has been the source of great controversy for many years. No one seriously doubts the *association* between *in utero* irradiation and childhood cancer; the debate is whether the radiation is *causative*, or whether it involves the selection of a particular group of children prone to cancer.

In a careful paper in 1997, Doll and Wakeford summarized all of the evidence for and against and came to the following conclusions:

- Low-dose irradiation of the fetus *in utero*, particularly in the last trimester, causes an increased risk of childhood malignancies.
- An obstetric x-ray examination results in a 40% increase in the risk of childhood cancer over the spontaneous level.
- Radiation doses of around 10 mGy (1 rad) increase the risk.
- The excess absolute risk is about 6% per gray.

TABLE 12.3. Childhood Cancer and Irradiation In Utero

Number of children with leukemia or cancer before age 10 y	7,649
Number x-rayed <i>in utero</i>	1,141
Number of matched controls	7,649
Number of controls irradiated <i>in utero</i>	774
Number of films	1 to 5
Fetal dose per film	0.46-0.2 rad (4.6-2 mGy)
Relative cancer risk estimate assuming radiation to be the causative agent	1.52

Based on Stewart A, Kneale GW: Radiation dose effects in relation to obstetric x-rays and childhood cancers. Lancet i:1185-1188, 1970.

### OCCUPATIONAL EXPOSURE OF WOMEN

The maximum permissible dose to the fetus during the entire gestation period from occupational exposure of the mother should not exceed 5 mSv (0.5 rem), with monthly exposure not exceeding 0.5 mSv (0.05 rem). This is the recommendation of the National Council on Radiological Protection and Measurements. Once a pregnancy is declared, the radi-

ation worker should be interviewed by the radiation safety officer or the chairman of the radiation safety committee to decide whether duties need to be changed or curtailed. There are only a few occupations in which there is the possibility of an unplanned radiation exposure, which, while not being more than that allowed a radiation worker, would exceed the more stringent limits suggested for the unborn child. The 5-mSv (0.5-rem) limit is based on the premise that the unborn child is sensitive to the production of defects and/or to an increased risk of leukemia, which is not warranted in the performance of the mother's occupation, although it may be acceptable in the course of delivering her health care.

### THE PREGNANT OR POTENTIALLY PREGNANT PATIENT

Most practicing radiologists at some time in their careers are faced with a patient who has discovered in retrospect that she was pregnant at a time at which extensive x-ray procedures were performed that involved the pelvis or lower abdomen.

The only completely satisfactory solution to this problem is to ensure that the situation never occurs in the first place. Patients should always be asked if they are, or may be, pregnant, and in the case of procedures involving larger doses of radiation to the pelvis, a pregnancy test may be in order.

Despite the best-laid plans and the most careful precautions, there still are occasional instances in which, because of clinical urgency or unusual accident, an early developing embryo is exposed to a substantial dose of radiation amounting to several centigrays (rads) or more. The first step is to estimate the dose involved. It is sometimes useful to solicit the help of an experienced medical physicist to make measurements in a phantom after carefully reconstructing the setup that was used. No dose level can be regarded as completely safe. Congenital abnormalities occur in 5 to 10% of the human population anyway, and so it is impossible in retrospect to attribute a given anomaly to a small dose of ra-

diation received by an embryo or fetus. All that can be said is that radiation increases the probability of an anomaly and that this increase is a function of dose.

The figure of 0.1 Gy (10 rad) often is mentioned as the dose to a developing embryo or fetus at a gestational age sensitive to the induction of congenital malformations, including reduced head diameter and mental retardation, above which a therapeutic abortion should be considered. This period extends from about 10 days to 26 weeks of gestation. The basis of this recommendation is as follows: The data from Japan for severe mental retardation could be interpreted as having a threshold, and the mechanism of the radiation effect is consistent with this conclusion. At the same time, the loss of IQ measured at 1 Gy (100 rad) would be undetectable if extrapolated linearly to 0.1 Gy (10 rad).

Not everyone would agree with this view, and the cut-off point is clearly not sharp. If a dose approaching this value has been given during the sensitive period, however, it is prudent to consider the relative merits of terminating the pregnancy in consultation with the referring physician as well as with the patient and her family. There are a number of factors to consider in conjunction with the dose. These include the hazard of the pregnancy to the expectant mother, the probability of future pregnancies, the extent to which the prospective parents want the unborn infant, their mental outlook on the possibility of a deformed child, and the ethnic and religious background of the family. The exact dose level at which it is justifiable to terminate the pregnancy may be flexible within broad limits around the guideline figure depending on a combination of these other circumstances.

There are special problems involved in the use of nuclear-medicine procedures in pregnant or potentially pregnant females. This is particularly true in the case of radionuclides that are able to cross the placenta. This topic is discussed in Chapter 14.

Table 12.4 is a historical summary of events in our gradual understanding of radiation effects on the developing embryo and fetus.

TABLE 12.4. *Major Events in Understanding Effects of Radiation on the Developing Embryo and Fetus*

Investigators	Year	Observations
Goldstein and Murphy	1929	Various abnormalities, including mental retardation and small head diameter in children born to mothers who received pelvic radiation therapy during pregnancy
Job et al.	1935	Recognition that different periods of gestation differ in radiosensitivity
Russell	1950	Nature of developmental abnormality determined by gestational age at exposure
Russell and Russell	1952	Clinical implications of irradiation in pregnancy
Plummer	1952	Mental retardation and microcephaly observed in children of atomic-bomb survivors
Stewart and Kneale	1952	Leukemia and childhood cancer in children irradiated in utero with diagnostic x-rays
Otake and Schull	1984	Mental retardation caused by irradiation at 8-15 weeks of pregnancy in Japanese survivors

#### SUMMARY OF PERTINENT CONCLUSIONS

- Moderate doses of radiation can produce catastrophic effects on the developing embryo and fetus.
- The effects depend on the stage of gestation, the dose, and also the dose rate.
- Gestation is divided into preimplantation, organogenesis, and the fetal period. In humans, these periods correspond to about 0 through 9 days, 10 days through 6 weeks, and 6 weeks through term, respectively.
- The principal effects of radiation on the developing embryo and fetus are growth retardation; embryonic, neonatal, or fetal death; congenital malformations; and functional impairment such as mental retardation.
- Irradiation during preimplantation leads to death of the embryo. Growth retardation or malformations are not seen in animals at this time. The human data are consistent with this conclusion.
- In animals, embryos exposed to radiation in early organogenesis exhibit the most severe intrauterine growth retardation, from which they can recover later (*i.e.*, temporary growth retardation). Irradiation in the fetal period leads to the greatest degree of permanent growth retardation.
- In animals, lethality from irradiation varies with stage of development. The embryonic 50% lethal dose is lowest during early preimplantation; at this stage, embryos killed by radiation suffer a prenatal death and are resorbed. In organogenesis, prenatal death is replaced by neonatal death—death at or about the time of birth. During the fetal stage the 50% lethal dose approaches that of the adult.
- In animals, the peak incidence of teratogenesis, or gross malformations, occurs if the fetus is irradiated in organogenesis.
- Contrary to what is observed in experimental animals, radiation-induced malformations of body structures other than the central nervous system are uncommon in the Japanese survivors irradiated *in utero*, although they have been reported in patients exposed to therapeutic doses of medical radiation.
- In the Japanese survivors, irradiation *in utero* resulted in small head size (microcephaly) and mental retardation.
- Mental retardation occurred primarily at 8 to 15 weeks of gestational age, with a smaller excess at 16 to 25 weeks. It is thought to be caused by radiation effects on cell migration within the brain.

- Cells killed before 8 weeks of gestational age cause small head size without mental retardation.
- Small head circumference was three times more common than mental retardation.
- Data on atomic-bomb survivors indicate that microcephaly can result from an air dose (kerma) of 0.1 to 0.19 Gy (10-19 rad)
- The incidence of severe mental retardation as a function of dose is reported to be apparently linear without threshold at 8 to 15 weeks, with a risk coefficient of 0.4 per Gy (0.4-100 rad). The incidence is about four times lower at 16 to 25 weeks. The data are consistent with a dose threshold of 0.12 to 0.2 Gy (12-20 rad).
- A variety of effects have been documented in experimental animals after irradiation during fetal stages, including effects on the hematopoietic system, liver, and kidney, all occurring, however, after quite high radiation doses.
- The effects on the developing gonads have been well documented both morphologically and functionally. Doses close to 1 Gy (100 rad) are needed to produce fertility changes in various species.
- There is an association between exposure to diagnostic x-rays *in utero* and the subsequent development of childhood malignancies.
- The original study of diagnostic x-ray exposure *in utero* and subsequent malignancies was done by Stewart and Kneale at Oxford University, but the same association was observed in the United States by MacMahon. If x-rays are the causative agent, these studies imply that radiation at low doses *in utero* increases the spontaneous cancer incidence in the first 10 to 15 years of life by a factor of 1.5 to 2.
- It has been argued for years whether radiation is the causative agent or whether there are other factors involved.
- Doll and Wakeford in 1997 summarized all of the evidence for and against and concluded that an obstetric x-ray examination, particularly in the third trimester, increased the risk of childhood cancer by 40%. The risk is increased by a dose of only 10 mGy (1 rad). The excess absolute risk is about 6% per gray, which is not very different from the risk estimates from the atomic-bomb survivors for adult exposure.
- Once a pregnancy is declared, the maximum permissible dose to the fetus is 0.5 mSv (0.05 rem) per month. Until a pregnancy is declared, no special limits apply to the mother other than those applicable to any radiation worker.
- Once a pregnancy is declared, the duties of a radiation worker should be reviewed to ensure that this limit is not exceeded.
- A dose of 0.1 Gy (10 rad) to the embryo during the sensitive period of gestation (10 days to 26 weeks) often is regarded as the cut-off point above which a therapeutic abortion should be considered to avoid the possibility of an anomalous child. The decision to terminate a pregnancy should be flexible and must depend on many factors in addition to dose.

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# 13

## Radiation Cataractogenesis

CATARACTS OF THE OCULAR LENS  
LENS OPACIFICATION IN EXPERIMENTAL ANIMALS  
CATARACTS IN HUMANS  
THE DEGREE OF OPACITY

THE LATENT PERIOD  
DOSE-RESPONSE RELATIONSHIP FOR CATARACTS IN HUMANS  
SUMMARY OF PERTINENT CONCLUSIONS

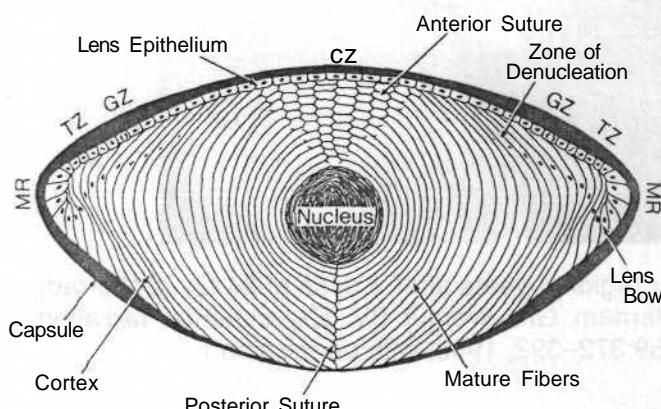
### CATARACTS OF THE OCULAR LENS

The word **cataract** is used to describe any detectable change of the normally transparent lens of the eye. The effect may vary from tiny flecks in the lens to complete opacification, resulting in total blindness. Cataracts are associated most usually with old age or less commonly with some abnormal metabolic disorder, chronic ocular infection, or trauma. It is also well known that sufficient exposure to ionizing radiations (such as x- or y-rays, charged particles, or neutrons) may cause a cataract.

The ocular lens is enclosed in a capsule (Fig. 13.1); the lens itself consists largely of

fiber cells and is covered with an epithelium anteriorly. The lens has no blood supply. Dividing cells are limited to the preequatorial region of the epithelium. The progeny of these mitotic cells differentiate into lens fibers and accrete at the equator.

Cell division continues throughout life, and so the lens may be regarded as a self-renewal tissue. It is, however, a most curious cellular system in that there appears to be no mechanism for cell removal. If dividing cells are injured by radiation, the resulting abnormal fibers are not removed from the lens but migrate toward the posterior pole; because they are not translucent, they constitute the beginning of a cataract.



**Figure 13.1.** Diagram of a sagittal section of a human lens illustrating the various cellular relationships. Cells are produced by mitosis in the germination zone of the epithelium (GZ). They begin to differentiate into lens fibers at the meridional rows (MR) and accumulate at the equator. Cells in the central zone (CZ) do not normally divide. (From Merriam GR, Worgul BV: Bull NY Acad Med 59:372-392, 1983)

### LENS OPACIFICATION IN EXPERIMENTAL ANIMALS

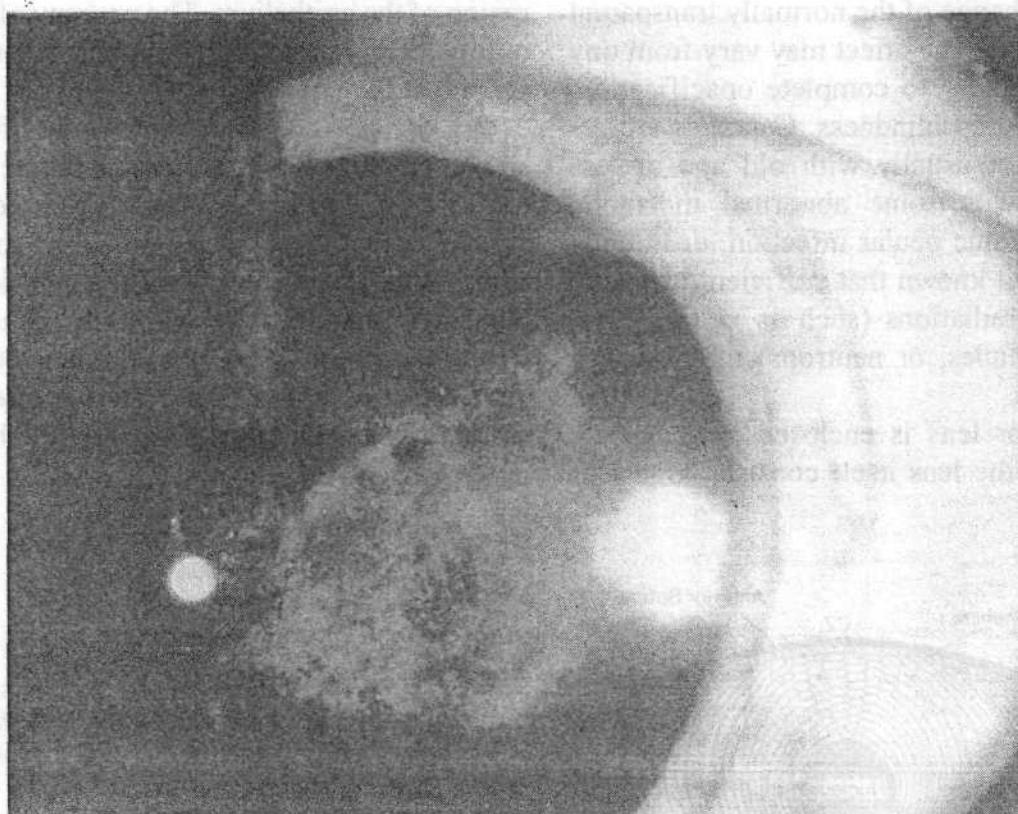
Some species of animals, especially the mouse, are very sensitive to radiation as far as lens opacification is concerned. A dose of a few centigray of x-rays or a fraction of a centigray of fast neutrons produces readily discernible changes in the lens. A large proportion of a mouse population naturally develops opacifications as they become older. As the dose is increased, the **latent period**, the time that elapses before an opacity of given severity is evident, becomes shorter. Put another way, radiation advances in time, a process that occurs normally.

Neutrons and other densely ionizing radiations are very effective at inducing cataracts, as evidenced by the number of physicists and engineers who developed cataracts as a result of

working around high-energy accelerators in the early days. The relative biologic effectiveness (RBE) of the fast neutrons is a strong function of dose, with a value of about 10 pertaining to high dose levels on the order of several gray (several hundreds of rad), relative to x-rays, but rising to 50 or more for small doses of a fraction of a centigray (rad). Worgul and his associates have reported similar RBEs for lens damage in rat eyes exposed to accelerated heavy ions. The increase in RBE at low doses is caused largely by a sharply declining effectiveness of x-rays with decreasing dose, rather than an increase in effect per unit dose of neutrons or charged particles.

### CATARACTS IN HUMANS

Radiologists have known for many years that the lens of the eye may be damaged by ra-



**Figure 13.2.** Cataract in the posterior subcapsular region 4 years after a dose of 24 Gy (2400 rad) of x-rays to a patient on radiotherapy. (From Merriam GR, Worgul BV: Experimental radiation cataract: Its clinical relevance. Bull NY Acad Med 59:372-392, 1983, with permission.)

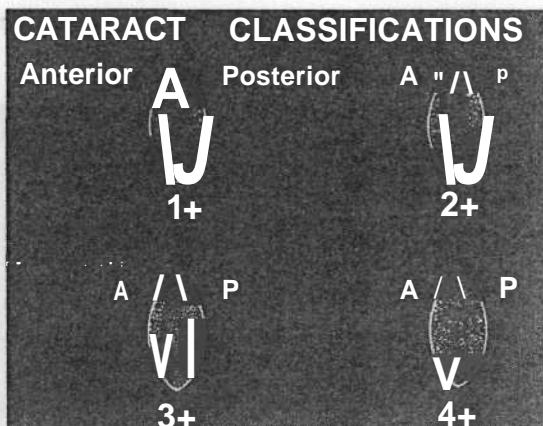
diation. A study of patients treated with x- or y-rays, in which a proportion of the dose reached the eye, has provided some insight into radiation cataractogenesis in humans. Figure 13.2 shows a typical cataract in a patient on radiotherapy. An early radiation cataract viewed through an ophthalmoscope may appear as a dot, usually situated at the posterior pole. As it enlarges, small granules and vacuoles appear around it. With further enlargement to the point at which the opacity is several millimeters in diameter, it may develop with a relatively clear center, so that it is shaped like a doughnut. At the same time, granular opacities and vacuoles may appear in the anterior subcapsular region, usually in the pupillary area. Depending on dose, the cataract frequently remains stationary at this stage, confined to the posterior subcapsular region. If it continues to progress, it becomes nonspecific and cannot be distinguished from other types of cataracts.

### THE DEGREE OF OPACITY

At low doses, the opacity may become stationary at a level that involves little or no impairment of vision. At higher doses, the opacity may progress until it results in a significant loss of vision. Of patients on radiotherapy who received low dose levels to the eye (2.2-6.5 Gy [220-650 rad]), only about 12% developed progressive opacities. Conversely, in higher-dose groups (6.5-11.5 Gy [650-1,150 rad]), only 12% had stationary opacities.

Figure 13.3 shows the system of cataract classification devised some years ago by Merriam and Focht. The accumulation of some opaque fibers at the posterior pole is labeled a 1+ cataract; as the severity of this opacity increases, and some impaired fibers show up in the anterior part of the lens, the score edges up progressively to 4+.

The severity of the cataract can be assessed quantitatively and objectively by using a Scheimpflug Imaging System. This device

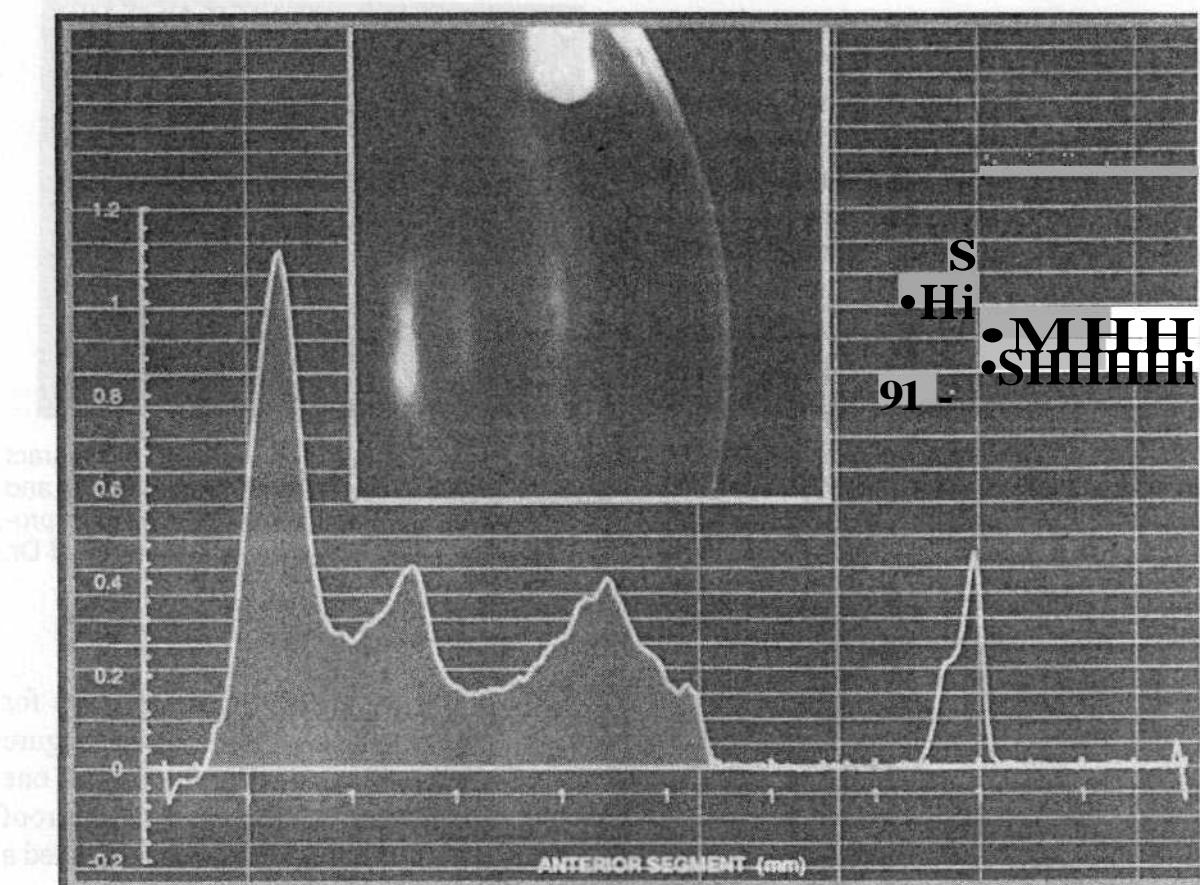


**Figure 13.3.** Illustrating the system of cataract classification devised by Merriam and Focht, and the arbitrary numerical scores assigned to progressive severities of cataracts. (Courtesy of Dr. Basil Worgul.)

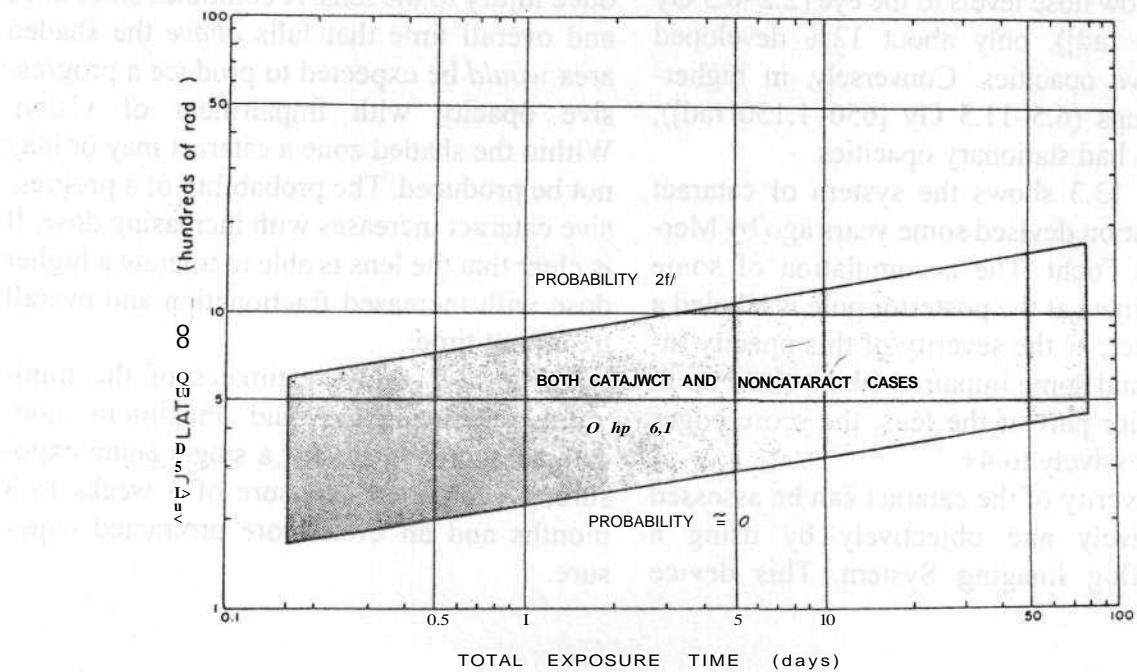
provides a distortion-free digitized image for densitometric analysis of the cataract. Figure 13.4 shows a cross-section of the lens of one of the "liquidators" who worked on the roof of the reactor at Chernobyl and accumulated a significant dose. The degree of opacity, measured with the Scheimpflug system, is shown in the lower panel.

Figure 13.5, deduced from accumulated clinical experience, represents a dose-time relationship for the production of cataracts. A combination of a dose and a treatment time that falls *below* the shaded band *does not* produce injury to the lens. A combination of dose and overall time that falls *above* the shaded area *would* be expected to produce a progressive opacity with impairment of vision. Within the shaded zone a cataract may or may not be produced. The probability of a progressive cataract increases with increasing dose. It is clear that the lens is able to tolerate a higher dose with increased fractionation and overall treatment time.

Table 13.1 shows estimates of the minimum cataractogenic and maximum non-cataractogenic doses for a single acute exposure, a protracted exposure of 3 weeks to 3 months and an even more protracted exposure.



**Figure 13.4. Top:** Photograph of the lens of a "liquidator" who worked on top of the reactor at Chernobyl and accumulated a substantial radiation dose. **Bottom:** Degree of opacity through the lens measured with the Scheimpflug Imaging System. This equipment gives a quantitative and objective assessment of the severity of the cataract. The area under the curve represents a densitometric reading of the lens. The region of greatest opacification is under the posterior capsule. (Courtesy of Dr. Basil Worgul.)



**TABLE 13.1.** Relation Between Overall Exposure Time and the Radiation Dose Needed to Produce a Cataract

Duration of Treatment	Minimum Cataractogenic Dose, Gy	Maximum Noncataractogenic Dose, Gy
Single	2.0	2.0
3 weeks-3 months	4.0	10.0
Over 3 months	5.5	10.5

### THE LATENT PERIOD

The time period between irradiation and the appearance of lens opacities in humans has been reported in the literature variously to be from 6 months to 35 years. In patients who had received 2.5 to 6.5 Gy (250-650 rad) the average latent period was about 8 years. At higher doses of between 6.51 to 11.5 Gy (651-1,150 rad), the average latent period was reduced to about 4 years. This and other evidence indicate that the latent period becomes shorter as dose is increased.

### DOSE-RESPONSE RELATIONSHIP FOR CATARACTS IN HUMANS

Merriam, Szechter, and Focht carefully reviewed the case histories of 233 patients on radiotherapy who received radiation to the lens of the eye and for whom dose estimates were available. Of these, 128 developed cataracts, 105 did not.

Britten and his colleagues reported 14 cases of radiation-induced cataracts in 38 patients treated with radon gold seed implants for tumors of the eyelid; in six visual acuity was seriously affected. These cataracts were thought to be progressive between 6 and 11 years after treatment. Doses were calculated to the center of the lens, and it appeared that

4,000R (about 40 Gy) produced a cataract in all cases, whereas 2,000R (about 20 Gy) resulted in no cataracts at all. In a parallel series treated by superficial x-rays, only one case of radiation cataract was observed in 57 patients treated; this was in the contralateral eye, which received a dose of 9.5 Gy (950 rad) in a single exposure, which presumably was transmitted through-the nose to the opposite eye. The lens dose on the treated side that was shielded with lead was only 0.16 Gy (16 rad).

Observations of the survivors of Hiroshima and Nagasaki have been consistent with the data from patients on radiotherapy. Large doses of radiation are required to produce vision-impairing cataracts. Physicists exposed to neutrons during the operation of cyclotron accelerators and survivors of reactor accidents also have developed cataracts, but the numbers are too small and the doses are not known with sufficient certainty to allow a meaningful construction of a dose-response relationship.

The available information appears to indicate the existence of a threshold for the induction of detectable lens opacification in humans. This does not exclude the possibility that the smallest doses do produce some damage, but in practical terms a dose of several grays (several hundred rads) is required to result in a demonstrable effect, and even larger

**Figure 13.5.** Time-dose relationship indicating radiation dosage for cataract production in humans with a probability between .0 and 1. A combination of a total dose and overall treatment time that falls above the shaded area would produce a progressive vision-impairing opacity. A dose and treatment time falling below the shaded area would not be expected to produce injury to the lens. Within the shaded area a cataract may or may not be produced; the probability increases with increasing dose. (From Merriam GR, Szechter A, Focht EF: The effects of ionizing radiations on the eye. *Front Radiat Ther Oncol* 6:346-385, 1972, with permission.)

doses to produce a cataract that impairs vision. Great care should be exercised in the use of neutrons and indeed all forms of high linear energy transfer radiations, because animal experiments indicate that they have a high RBE for lens opacification.

Radiation-induced cataracts are considered to be a deterministic (nonstochastic) late effect, because there is a practical threshold dose below which they do not occur, and above the threshold the severity of the biologic response is dose-related.

### SUMMARY OF PERTINENT CONCLUSIONS

- A cataract is an opacification of the normally transparent lens of the eye.
- Dividing cells are limited to the preequatorial region of the epithelium. Progeny of these mitotic cells differentiate into lens fibers and accrete at the equator. It is the failure of these cells to differentiate correctly that leads to a cataract, whether spontaneous or radiation-induced.
- The minimum dose required to produce a progressive cataract is about 2 Gy (200 rad) in a single exposure; larger doses are necessary in a fractionated or protracted exposure. The minimum dose increases to 4 Gy (400 rad) spread over 3 weeks to 3 months and 5.5 Gy (550 rad) for more than 3 months.
- The latent period between irradiation and the appearance of a lens opacity is dose-related. The latency is about 8 years after exposure to a dose in the range of 2.5 to 6.5 Gy (250-650 rad).
- The RBE of neutrons or heavy ions is about 20 at high doses but rises to 50 or more for small doses.
- A radiation-induced cataract is a deterministic (nonstochastic) late effect. There is a practical threshold dose below which cataracts are not produced, and above this threshold the severity of the biologic response is dose-related.

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# Doses and Risks in Diagnostic Radiology, Interventional Radiology and Cardiology, and Nuclear Medicine

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DOSES FROM NATURAL BACKGROUND  
RADIATION  
COMPARISON OF RADIATION DOSES  
FROM NATURAL SOURCES AND  
HUMAN ACTIVITIES  
DIAGNOSTIC RADIOLOGY

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INTERVENTIONAL RADIOLOGY AND  
CARDIOLOGY  
NUCLEAR MEDICINE  
MEDICAL IRRADIATION OF CHILDREN  
AND PREGNANT WOMEN

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This chapter is to review the doses involved, and to estimate the associated risks, in radiology, cardiology, and nuclear medicine. But first, in order to put things in perspective, the radiation doses from natural background are summarized. This usually is regarded as an important benchmark, since life on earth has evolved in a continuous radiation background.

## DOSES FROM NATURAL BACKGROUND RADIATION

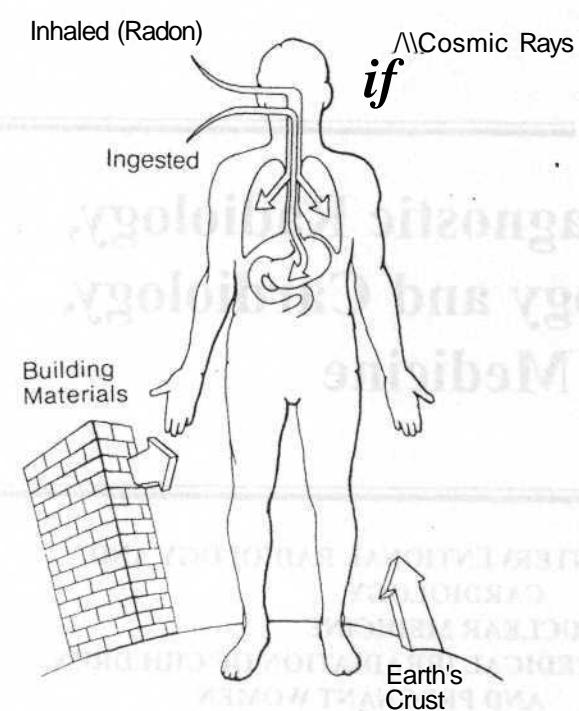
**Natural sources** of radiation include cosmic rays from outer space, terrestrial radiation from natural radioactive materials in the ground, and radiation from radionuclides naturally present in the body, inhaled, or ingested. The sources of natural background radiation are illustrated in Figure 14.1.

**Enhanced natural sources** are sources that are natural in origin but to which exposure is increased as a result of human activity (inadvertent or otherwise). Examples include

air travel at high altitude, which increases cosmic-ray levels, arid movement of radionuclides on the ground in phosphate mining, which can increase the terrestrial component to persons living in houses built on waste landfills. Radon exposure indoors might be considered in some instances to be an enhanced natural source, inasmuch as it is not natural to live in an insulated house. In a sense, also, all operations of the nuclear fuel cycle, starting with mining, involve natural radionuclides, but these are more generally classified as a consequence of human activity.

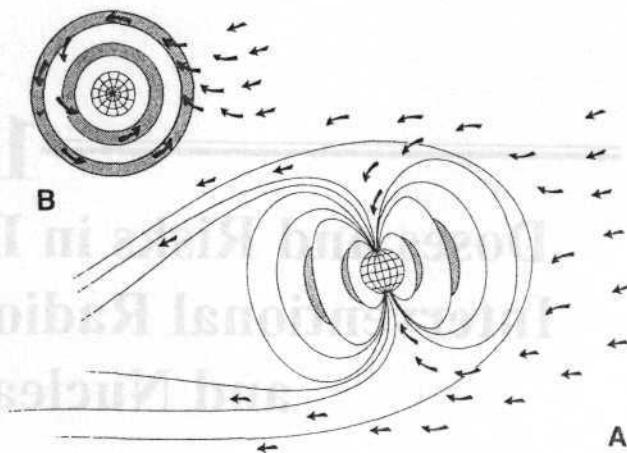
### Cosmic Radiation

Cosmic rays are made up of radiations originating from outside the solar system and from charged particles (largely protons) emanating from the surface of the sun. The intensity of cosmic rays arriving at the earth's surface varies with both latitude and altitude above sea level. The variation with latitude is a consequence of the magnetic properties of the earth:



**Figure 14.1.** Three principal components of natural background radiation: (1) cosmic rays from solar flares in the sun or from outer space; (2) ingested radioactivity, principally potassium-40 in food, and inhaled radioactivity, principally radon; and (3) radiation from the earth's crust, which in practice means from building materials, because most persons spend much of their lives indoors.

Cosmic rays are charged particles that are deflected away from the equator and tunneled into the poles. This is illustrated in Figure 14.2. The aurora borealis, or northern lights, results from charged particles spiraling down the lines of magnetic field into the polar regions. Consequently, cosmic-ray intensity is least in equatorial regions and rises towards the poles. There is an even larger variation in cosmic-ray intensity with altitude, because at high elevations above sea level there is less atmosphere to absorb the cosmic rays, and so their intensity is greater. For example, the cosmic-ray annual equivalent dose in the United States is about 0.26 mSv (26 mrem) at sea level. This essentially doubles for each 2,000-m increase in altitude in the lower atmosphere, so that in Denver, Colorado, the annual effective dose from cosmic radiation is about 0.5 mSv (50 mrem). Long flights at high altitudes involve some

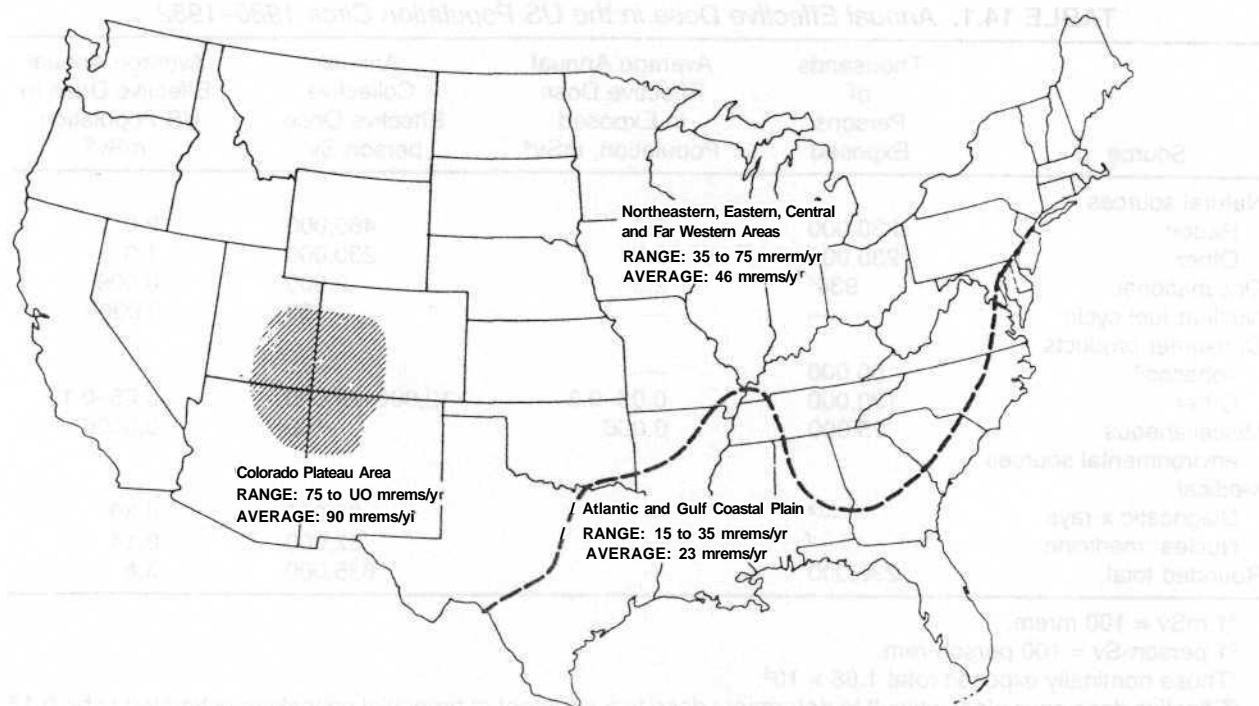


**Figure 14.2. A:** The earth behaves like a giant magnet. Showers of charged particles from solar events on the surface of the sun are deflected away from the equator by the magnetic field of the earth; most miss the earth altogether; others are funneled into the polar regions. This explains why cosmic-ray dose is low near the equator and high in the polar regions. It is also the basis of the aurora borealis, or northern lights; intense showers of cosmic-ray particles spiral down the lines of magnetic field into the poles. **B:** Viewed from above the poles, the earth is ringed with lines of magnetic field that form regions of high radiation dose known as the *van Allen belts*. Man could not live for long in the dose rates characteristic of these belts. To leave earth, spaceships pass quickly through the *van Allen belts*; the space shuttle orbits well below them.

dose, too. For example, the extra dose from cosmic rays received by a passenger on a commercial flight flying from the United States to Europe is about 0.05 mSv (5 mrem). Flight crews on northerly routes accumulate larger doses than most radiology staff in hospitals; in fact, airline crews are already classified as radiation workers in Europe, but that is not yet the case in the United States.

#### Natural Radioactivity in the Earth's Crust

Naturally occurring radioactive materials are widely distributed throughout the earth's crust, and as a consequence, humans are exposed to the  $\gamma$ -rays from them. There is a big variation between areas such as Colorado, where the rocks and soil contain radioactive thorium and uranium, and areas such as the



**Figure 14.3.** The variation of the component of natural background radiation originating from the earth's crust. (National Research Council, 1980. Committee on the Biological Effects on Ionizing Radiations (BEIR III). The Effects on Populations of Exposure to Low Levels of Ionizing Radiation; Washington, D.C. National Academy Press.)

Atlantic seaboard, where radioactivity is low. This is shown in Figure 14.3.

### Internal Exposure

Small traces of radioactive materials are normally present in the human body, ingested from the tiny quantities present in food supplies or inhaled as airborne particles. Radioactive thorium, radium, and lead can be detected in most persons, but the amounts are small and variable and the figure usually quoted for the dose rate resulting from these deposits is less than 10 ( $\mu\text{Sv}/\text{y}$  (1 mrem/y)). Only radioactive potassium-40 makes an appreciable contribution to human exposure from ingestion. The dose rate is about 0.2  $\text{mSv}/\text{y}$  (20 mrem/y), which cannot be ignored as a source of mutations in humans.

The biggest source of natural background radiation is radon gas, which seeps into the basement of houses from rocks underground. It is a decay product in the uranium series. Radon itself is a noble gas that does little harm, but in the confined space of an underground mine or

the basement of a house, it decays with a 3-day half-life to form solid progeny that stick to dust particles and if inhaled become lodged on the surface of the bronchus or lung. Radon progeny emit  $\alpha$ -particles that, it is believed, are responsible for lung cancer. Radon levels in houses vary enormously, but the average concentration in the United States appears to be about 37  $\text{Bq}/\text{L}$  (1  $\text{pCi}/\text{L}$ ) in the living area and much more in the basement. This translates into an annual effective dose (Table 14.1) of about 2  $\text{mSv}$  (200 mrem). Only the lungs are irradiated by this source, but  $\alpha$ -particles are highly effective and have a radiation weighting factor of 20. There is no question that radon is by far the largest component of natural background radiation. The Environmental Protection Agency action level for radon is 148  $\text{MBq}/\text{L}$  (4  $\text{pCi}/\text{L}$ ); remedial action is suggested for houses above this level. The Committee on the Biological Effects of Ionizing Radiation of the National Academy of Sciences estimates that radon may be responsible for between 15,400 and 21,800 lung cancer deaths per year in the United States.

**TABLE 14.1.** Annual Effective Dose in the US Population Circa 1980-1982

Source	Thousands of Persons Exposed	Average Annual Effective Dose in Exposed Population, mSv <sup>a</sup>	Annual Collective Effective Dose, person-Sv <sup>b</sup>	Average Annual Effective Dose in US Population, mSv <sup>c</sup>
Natural sources				
Radon	230,000	2.0	460,000	2.0
Other	230,000	1.0	230,000	1.0
Occupational	930 <sup>c</sup>	2.3	2,000	0.009
Nuclear fuel cycle	—	—	136	0.0005
Consumer products				
Tobacco <sup>d</sup>	50,000	—	—	—
Other	120,000	0.05-0.3	12,000-29,000	0.05-0.13
Miscellaneous environmental sources	-25,000	0.006	160	0.0006
Medical				
Diagnostic x-rays	— <sup>e</sup>	—	91,000	0.39
Nuclear medicine	— <sup>f</sup>	—	32,000	0.14
Rounded total	230,000	—	835,000	3.6

<sup>a</sup>1 mSv = 100 mrem.<sup>b</sup>1 person-Sv = 100 person-rem.Those nominally exposed total  $1.68 \times 10^6$ .<sup>c</sup>Effective dose equivalent difficult to determine; dose to a segment of bronchial epithelium estimated to be 0.16 Sv/y (16 rem/y).<sup>d</sup>Number of persons exposed is not known. Number of examinations was 180 million and effective dose per examination 500 nSv.<sup>e</sup>Number of persons exposed is not known. Number of examinations was 7.4 million and effective dose per examination 4,300  $\mu$ Sv.

Data from National Council on Radiation Protection and Measurements: Exposure of the Population in the United States to Ionizing Radiation. Report No. 93. Bethesda, MD, NCRP, 1987.

### Areas of High Natural Background

There are several inhabited areas of the world in which background radiation is considerably elevated because of radioactivity in rocks or soil or in building materials from which houses are made. These areas are in Brazil, France, India, Niue Island in the Pacific, and Egypt.

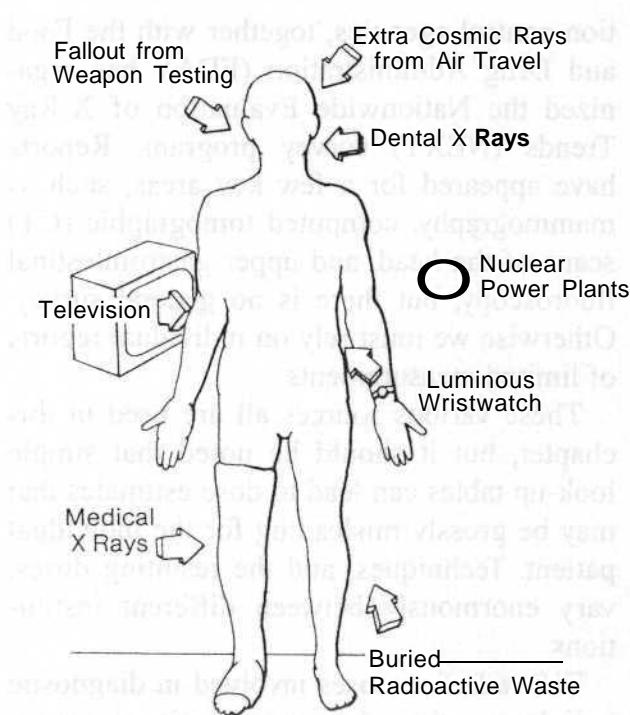
In Brazil, some 30,000 persons who live in coastal areas are exposed to dose rates of 5 mSv/y (500 mrem/y). About one sixth of the population of France, largely in the Burgundy wine-growing district, lives in areas in which the rocks are principally granite and receive 1.8 to 3.5 mSv/y (180-350 mrem/y) from background radiation. Undoubtedly, the highest background is in Kerala, India, where more than 100,000 persons receive an annual dose of about 13 mSv (1,300 mrem).

Many studies have been made of these human populations, who have lived for many generations in areas of high natural background radiation. So far, no excess incidence

of cancer or hereditary anomalies have been observed that can reasonably be attributed to the radiation. Such studies, of course, are beset with difficulties. Nevertheless, in spite of the obvious problems involved in making comparisons, it is an important and significant fact that human populations who have lived for generations at levels of background radiation that differ by an order of magnitude do not show noticeable differences in the incidence of cancer or genetic disorders. This is the basis for believing that as long as manmade radiation does not exceed the average background value, it is unlikely to produce any detectable deleterious effects on the world's population.

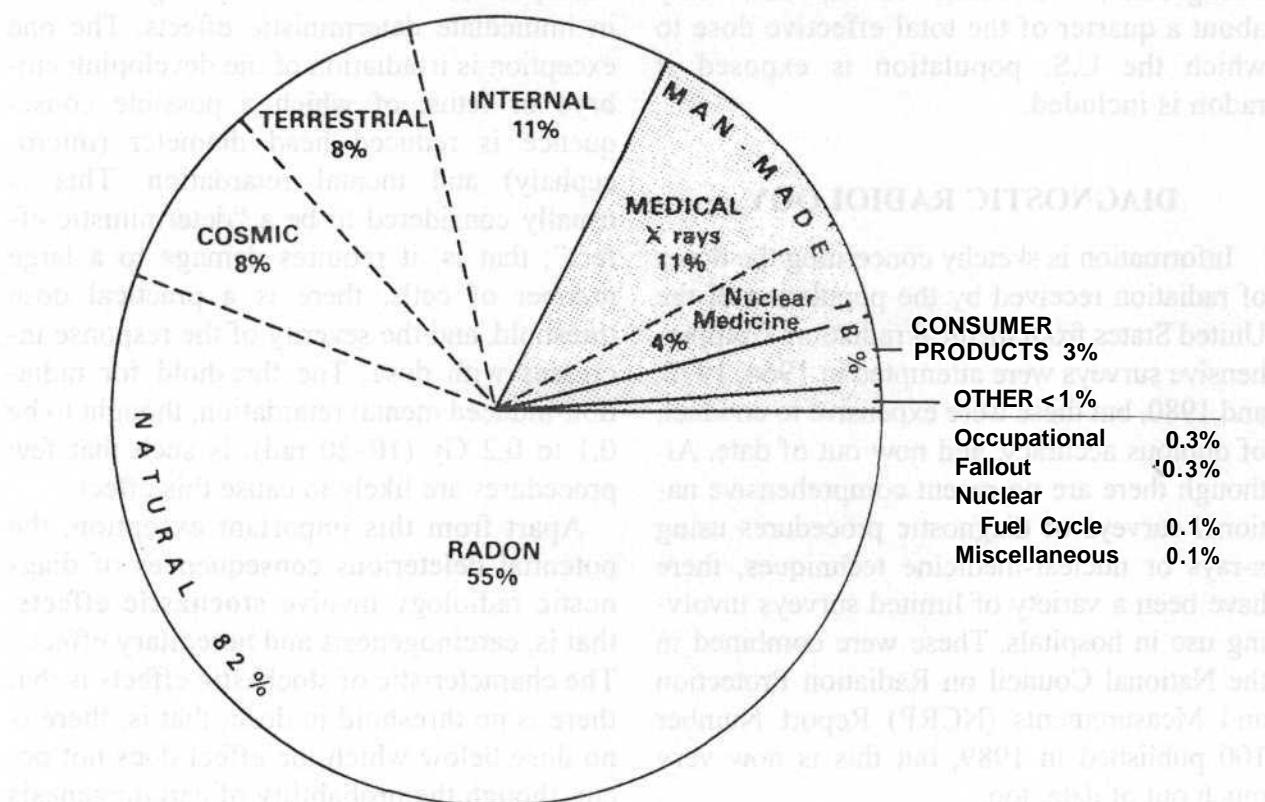
### COMPARISON OF RADIATION DOSES FROM NATURAL SOURCES AND HUMAN ACTIVITIES

In addition to natural background radiation, the human population is exposed to a variety of sources of radiation resulting from human activity, as illustrated in Figure 14.4.



Radiation doses to the U.S. population from the various sources are summarized in Table 14.1. The average dose is about 3.6 mSv (360 mrem) from all sources, which is a large excess over the background equivalent dose of 0.12 mSv (12 mrem). This is due to the significant contributions from medical procedures and consumer products.

**Figure 14.4.** The various sources of radiation resulting from human activity to which the human population is exposed. In developed countries the effective dose is dominated by medical radiation.



**Figure 14.5.** This pie diagram, which appeared in 1987, showed for the first time that the average effective dose to the population of the United States is dominated by indoor radon daughter products. The effective dose, of course, is the dose in grays (or rads), multiplied by the radiation weighting factor, which is 20 for the  $\alpha$ -particles emitted by radon daughter products, and multiplied by the tissue weighting factor, which is about 0.12 for the lungs. The annual effective dose to the U.S. population is about 3.6 mSv (360 mrem). More than one half of this is a result of radon, and altogether 82% comes from natural sources. Medical x-rays contribute only 11% and nuclear medicine 4%. (From National Council on Radiation Protection and Measurements: Ionizing Radiation Exposure of the Population of the United States. NCRP Report 93, Bethesda, MD, 1987.)

Radiation doses to the U.S. population from all sources are summarized in Figure 14.5 and in somewhat more detail in Table 14.1. The average annual effective dose\* from all sources amounts to 3.6 mSv (360 mrem). There is a large extra dose to the bronchial epithelium in smokers from naturally occurring radionuclides in tobacco products, but it is difficult to estimate with any precision.

There are several obvious conclusions to be drawn from this summary of doses from various sources. First, radon represents the largest source of radiation to the U.S. population. Second, medical diagnostic x-rays represent by far the largest source of radiation resulting from human activities. Third, the overall effective dose from medical radiation is about equal to that from natural background, excluding radon, and only about a quarter of the total effective dose to which the U.S. population is exposed if radon is included.

## DIAGNOSTIC RADIOLOGY

Information is sketchy concerning the doses of radiation received by the population of the United States from medical radiation. Comprehensive surveys were attempted in 1964, 1970, and 1980, but these were expensive to conduct, of dubious accuracy, and now out of date. Although there are no recent comprehensive national surveys of diagnostic procedures using x-rays or nuclear-medicine techniques, there have been a variety of limited surveys involving use in hospitals. These were combined in the National Council on Radiation Protection and Measurements (NCRP) Report Number 100 published in 1989, but this is now very much out of date, too.

In more recent years, the Conference of Radiation Control Program Directors, the umbrella organization for state and local radi-

tion control agencies, together with the Food and Drug Administration (FDA), has organized the Nationwide Evaluation of X-Ray Trends (NEXT) survey program. Reports have appeared for a few key areas, such as mammography, computed tomographic (CT) scans of the head, and upper gastrointestinal fluoroscopy, but there is no general survey. Otherwise we must rely on individual reports of limited measurements.

These various sources all are used in this chapter, but it should be noted that simple look-up tables can lead to dose estimates that may be grossly misleading for the individual patient. Techniques, and the resulting doses, vary enormously between different institutions.

The radiation doses involved in diagnostic radiology, other than interventional procedures, are seldom sufficiently large to result in immediate deterministic effects. The one exception is irradiation of the developing embryo or fetus, of which a possible consequence is reduced head diameter (microcephaly) and mental retardation. This is usually considered to be a "deterministic effect"; that is, it requires damage to a large number of cells, there is a practical dose threshold, and the severity of the response increases with dose. The threshold for radiation-induced mental retardation, thought to be 0.1 to 0.2 Gy (10-20 rad), is such that few procedures are likely to cause this effect.

Apart from this important exception, the potential deleterious consequences of diagnostic radiology involve **stochastic effects**, that is, carcinogenesis and hereditary effects. The characteristic of stochastic effects is that there is no threshold in dose; that is, there is no dose below which the effect does not occur, though the probability of carcinogenesis or hereditary effects increases with dose. As a consequence, absorbed dose to a limited portion of an individual's body does not by itself provide the overall perspective on risk associated with a given procedure. Effective dose is a more relevant quantity; it takes into account the tissues and organs irradiated, as

\*For the reader unfamiliar with terms such as *effective dose*, it may be necessary to first read the definitions in Chapter 15.

well as the dose involved. This is important, because some tissues and organs are more susceptible than others to radiation. Effective dose is discussed in detail in the chapter on radiation protection (Chapter 15). The technical definition of effective dose is the sum of the equivalent doses to each tissue and organ exposed, multiplied by the appropriate tissue weighting factors. What this amounts to in simpler terms is that effective dose is the whole-body dose of x-rays that would have to be delivered to produce the same stochastic risk as the partial-body dose that actually was delivered. This quantity provides an easy assessment of overall risk and makes comparison of risks much simpler; for example, risk from a diagnostic examination is more readily compared with that from background radiation if effective dose is quoted. If absorbed dose is in grays, the effective dose is in sieverts; if dose is in rads, effective dose is in rems. Many recent reports in the literature use effective dose in discussing the potential consequences of diagnostic radiology.

Last, but not least, the overall population impact of diagnostic radiology can be assessed in terms of the **collective effective dose**, the product of effective dose and the number of individuals exposed.

These three quantities, dose, effective dose, and collective effective dose, are discussed in turn.

#### Dose

Table 14.2 is a summary of entrance skin exposures, as well as organ doses, characteristic of a representative sample of standard diagnostic procedures. The data do not contain any big surprises. As would be expected, radiographs of the lumbar spine, barium enema series, and upper gastrointestinal series involve substantial doses of radiation, because of the need to penetrate these thick and dense regions of the body. These are the procedures, too, that inevitably lead to large gonadal doses. Entrance skin exposures from exami-

nations of the chest, abdomen, and lower spine are summarized in Table 14.3; these data come from the NEXT series. Doses involved in upper gastrointestinal fluoroscopy, also measured in the NEXT series, are shown in Table 14.4.

Some of the largest doses in diagnostic radiology are associated with fluoroscopy. In this case, the dose rate is greatest at the skin, where the x-ray beam first enters the patient. Although dose rates in the literature are now reported in the new SI unit of milligray per minute, existing regulations still specify limits in terms of an exposure rate (roentgen per minute). The entrance exposure limit for standard operation of a fluoroscope is 10 R/min (100 mGy/min). Some fluoroscopes are equipped with a high-output or "boost" mode, and the limit for operation in this mode on state-of-the-art equipment is 20 R/min (200 mGy/min). There is no limit on entrance exposure rate during any type of recorded fluoroscopy, such as cinefluorography or digital acquisitions.

A typical fluoroscopic entrance exposure rate for a man of medium build is approximately 3 R/min (30 mGy/min). Much higher dose rates may be encountered during recorded interventional and cardiac catheterization studies, such as those that involve a series of multiple, still-frame image acquisitions.

The number of CT scanners in clinical use has risen steadily over the years, reaching a global total in 1997 of about 20,000 units, with an associated annual total of some 67 million CT procedures. The distribution of scanners in the world is far from uniform, as shown by Table 14.5. By far the largest number of scanners per million population is found in the United States, and this is reflected in the highest number of annual procedures per thousand population. These figures in Table 14.5 are from the mid-1990s but are already out of date, because the use of CT continues to grow rapidly.

Several surveys have been completed of the doses of radiation resulting from CT scans of

TABLE 14.2. *Entrance Skin Exposure and Absorbed Doses to Various Organs From Radiographic Studies in Adults\**

Examination and View	Free-in-air Exposure at Skin Entrance, mR	Dose, mGy (mrads)						
		Active	Bone Marrow	Thyroid	Breast	Lungs	Ovaries	Testes
Chest								
PA	20		0.02 (2)	0.01 (1)	0.01 (1)	0.07 (7)	N	N
Lateral	65		0.02 (2)	0.07 (7)	0.15 (15)	0.12 (12)	N	N
Series	—		0.04 (4)	0.07 (7)	0.16(16)	0.19(19)	N	N
Skull								
AP	330		0.08 (8)	0.06 (6)	—	N	N	N
Lateral	190		0.05 (5)	0.21 (21)	—	N	N	N
Series	—		0.24 (24)	0.34 (34)	—	0.01 (1)	N	N
Cervical spine								
AP	150		0.02 (2)	1.00 (100)	—	0.02 (2)	N	N
Lateral	100		0.02 (2)	0.06 (6)	—	0.02 (2)	N	N
Series	—		0.09 (9)	2.60 (260)	—	0.11 (11)	N	N
Thoracic spine								
AP	280		0.05 (5)	0.25 (25)	0.95 (95)	0.35 (35)	N	N
Lateral	630		0.12 (12)	0.05 (5)	0.05 (5)	0.75 (75)	N	N
Series	—		0.17 (17)	0.30 (30)	1.00(100)	1.10 (110)	N	N
Lumbar spine								
AP	640		0.18 (18)	N	—	0.40 (40)	1.10 (110)	0.02 (2)
Lateral	2300		0.44 (44)	N	—	0.30 (30)	0.90 (90)	0.02 (2)
Series	—		1.10 (110)	N	—	1.70 (170)	3.70 (370)	0.06 (6)
Urography								
KUB (AP)	600		0.20 (20)	N	—	0.07 (7)	1.30 (130)	0.10 (10)
Series	—		0.90 (90)	N	—	0.27 (27)	5.50 (550)	0.40 (40)
Series + 4 tomograms	—		1.70 (170)	N	—	0.54 (54)	6.50 (650)	0.50 (50)
Mammography <sup>7</sup>	—	—	—	—	3.60 (360)	—	—	—
Upper gastrointestinal series	—	3.00 (300)	0.03 (3)	0.50 (50)	1.00 (100)	12.00 (1,200)	0.80 (80)	
Barium enema series	—	5.20 (520)	N	—	—	—	—	

Values given are exposures and doses received by some patients at some facilities. Values can be much higher or lower depending on patient size, the technology employed, and the examination protocols established by the radiologist. —, No estimate is made. N, negligible dose (<0.01 mGy [<1 mrads]).

<sup>7</sup>Two-view screening with film-screen grid.

Adapted from Wagner LK: Radiation Bioeffects and Management Test and Syllabus. Reston, VA, American College of Radiology, 1991, with permission.

**TABLE 14.3.** Mean Entrance Skin Exposure Values for Various Procedures from the 1994 and 1995 Nationwide Evaluation of X-Ray Trends Surveys

Year	Procedure	Air Kerma, mGy	Exposure, mR
1994	PA chest	0.141	16.1
1995	Abdominal exam	2.86	326
1995	LS spine exam	3.24	370

Courtesy of Dr. David Spelic, US Food and Drug Administration, Division of Mammography Quality and Radiation Programs.

**TABLE 14.4.** Nationwide Evaluation of X-Ray Trends (NEXT) Survey of Upper Gastrointestinal Fluoroscopy Examinations

	With Phantom	Phantom and Simulated Barium	Unit Driven to Maximum
Entrance Air Kerma Rate, mGy/min			
Average	38	56	59
Maximum	130	160	160
Minimum	10	10	10
Mean Single Spot Film Exposure, mGy			
Hospitals	2.1	19	
Nonhospitals	4.0	22	

the head. The 1990 NEXT survey evaluated some 252 CT scanning systems and found that the average central dose from a series of contiguous slices through the head was 34 to 55 mGy (3.4-5.5 rad); more details are included in Table 14.6. A similar survey of 14 CT scanners in Australia, evaluating doses from a series of 10 contiguous 10-mm slices, essentially all of the head, quoted an average dose of 45.8

**TABLE 14.5.** Global Activity in Computed Tomography for 1995

Region	Scanners per Million Population	Annual Procedures per Thousand Population
World	3.5	11
United States	26.4	91
European Union	<b>10.1</b>	33
France	7.7	33
Germany	16.6	53
Italy	9.6	30
Spain	5.7	15
United Kingdom	6.2	21

Adapted from Bahador B: London, FT Pharmaceutical and Health Care Publishing, London, 1996, with permission.

mGy (4.58 rad). Both surveys emphasized wide variations in dose with different model scanners and different techniques.

In general, the multiple scan average dose ranges are 40 to 60 mGy (4-6 rad) for head scans and 10 to 40 mGy (1-4 rad) for body scans. Patient dose also is incurred during acquisition of a scout scan. The CT scanner is used to acquire a scout planar image similar to that obtained in radiography. A scout scan usually results in a surface dose of approximately 1 mGy (100 mrad).

### Effective Dose

Many recent surveys of doses in diagnostic radiology emphasize the effective dose, because this is related to the risk of stochastic effects such as the induction of cancer or hereditary effects.

**TABLE 14.6.** Nationwide Evaluation of X-Ray Trends (NEXT) Survey of CT Examinations of the Head: Values for CT Systems with 10 or More Units in 1990

Manufacturer	Model	Multiple Scan Average Dose (Range), mGy <sup>a</sup>	Average, mA
GE	8800	42(17-119)	586
	9800	47 (20-140)	385
Philips	60	35(23-61)	348
Picker	1200	49 (23-70)	333
Siemens	DR	34 (19-66)	442

<sup>a</sup>Doses in mGy can be converted to mrad by multiplying by 100.

From Conway BJ, McCrohan JL, Antonsen RG, Reuter FG, Slayton RJ, Suleiman OH: Average radiation dose in standard CT examinations of the head: Results of the 1990 NEXT survey. Radiology 184:135-140, 1992, with permission.

**TABLE 14.7.** Effective Doses for Common Diagnostic Procedures (United States)

	ESAK, mGy	Entrance Skin Exposure, mR	EFFECTIVE DOSE, mSv (mrem)	
			Male	Female
Chest (PA)	0.18	20	0.03 (3)	0.03 (3)
Chest (lateral)	0.57	65	0.05 (5)	0.08 (8)
Skull (AP)	2.9	330	0.04 (4)	0.04 (4)
Skull (lateral)	1.5	166	0.02 (2)	0.02 (2)
C-spine (AP)	1.3	150	0.05 (5)	0.05 (5)
C-spine (lateral)	0.88	100	0.02 (2)	0.02 (2)
T-spine (AP)	2.5	280	0.27 (27)	0.54 (54)
T-spine (lateral)	6.0	680	0.25 (25)	0.27 (27)
L-spine (AP)	5.6	640	0.40 (40)	0.78 (78)
L-spine (lateral)	20	2300	0.53 (53)	0.84 (84)
Abdomen (AP)	5.3	600	0.37 (37)	0.73 (73)

Entrance skin exposure values taken from Wagner LK: Radiation Bioeffects and Management Test and Sylabus. Reston, VA, American College of Radiology, 1991.

Effective doses calculated by Dr. Beth A. Schueler using Rosenstein M: Handbook of Selected Tissue Doses for Projections Common in Diagnostic Radiology. HEW (FDA) Publication 89-8031 for organ doses (HVL assumed to be 3.0 mm Ae at 80 kVp, our kVp used for exam kVp settings) and ICRP: Recommendations of the ICRP. Publication 26. 1977 for risk weighting factors.

Table 14.7 shows the results of a study conducted in the United States by the FDA, listing the entrance skin exposure and the corresponding effective dose resulting from a representative sample of diagnostic procedures. Comparable data from the United Kingdom are shown in Table 14.8; this table also includes some information related to CT scans. Table 14.9 summa-

rizes similar data from Canada, which also include some measurements for positron emission tomographic (PET) scans for comparison,

It is not difficult to understand why CT scans involve relatively larger effective doses, because larger volumes of tissue are exposed to higher doses than with common x-rays. Table 14.10 dates from 1989 and so is

**TABLE 14.8.** Typical Effective Doses to Adult Patients in the 1990s (United Kingdom)

Examination	Typical Effective Dose, mSv
Single Radiographs	
Skull AP or PA	0.03
Skull LAT	0.01
Chest PA	0.02
Chest LAT	0.04
Thoracic spine AP	0.4
Thoracic spine LAT	0.3
Lumbar spine AP	0.7
Lumbar spine LAT	0.3
Lumbar spine LSJ	0.3
Abdomen AP	0.7
Pelvis AP	0.7
Complete Examinations	
IVU(6films)	2.5
Barium swallow (24 spot images, 106 s fluoro.)	1.5
Barium meal (11 spot images, 121 s fluoro.)	3
Barium follow (4 spot images, 78 s fluoro.)	3
Barium enema (10 spot images, 137 s fluoro.)	7
CT head	2
CT chest	8
CT abdomen	10
CT pelvis	10

From Wall BF, Hart D: Revised radiation doses for Typical x-ray examinations [commentary]. Br J Radiol 70:437-439, 1997, with permission.

TABLE 14.9. *Typical Effective Dose Values for Diagnostic Procedures (Canada)*

Procedure	Typical Effective Dose, mSv
Chest radiograph	0.02-0.05
Skull radiograph	0.15
Thoracic spine radiograph	0.90
Pelvic/abdominal radiograph	1.3
Head computed tomographic scan	2.0
Average positron emission tomographic scan (range)	3.9 (1.0-8.9)
Intravenous urogram	4.4
Average $^{99m}\text{Tc}$ radionuclide scan (range)	3.8-7.7
Body computed tomographic scan	6-16

Adapted from Huda W, Lettle B, Sutherland JB: J Can Assoc Radiol 40:3-4, 1989, with permission.

somewhat dated, but it represents an extensive U.K. survey of CT scans of various regions of the body, quoting both organ doses and effective doses. The effective dose varies from a low of 0.34 mSv (34 mrem) if only part of the head is exposed to a high of 7.8 mSv (780 mrem) for a routine multiple-slice chest CT scan. Table 14.11 compares effective doses in the UK for 1989 with comparable data from Wales in the mid-1990s; as expected, the effective dose per procedure does not change much with time. It is the frequency of use of CT imaging that is increasing steadily, and data on this in the United States are sparse.

Table 14.12 shows effective doses to patients undergoing abdominal CT examinations and makes the interesting point that effective doses are 50% larger in children younger than 10 years of age than in adults; this is presumably a consequence of the fact that critical organs are closer together in small children. Because children are two to three times more sensitive to the carcinogenic effect of x-rays than are adults, it is important that facilities reduce their doses for CT scanning in children.

The next two tables address special and very different circumstances. Table 14.13 compares the effective dose from cerebral an-

TABLE 14.10. *Mean Values of Patient Dose and Effective Dose from Computed Tomographic Examinations in the United Kingdom for 1989*

Examination	Organ Dose, mGy				
	Eyes	Uterus	Ovaries	Testes	Effective Dose, mSv
Routine head	50	—	0	0	1.8
Posterior fossa	53	—	—	0	0.72
Pituitary	60	—	—	0	0.57
Internal auditory meatus	2.6	—	0	0	0.35
Orbits	50	—	—	0	0.64
Facial bones	9.0	—	—	0	0.68
Cervical spine	0.62	—	—	0	2.6
Thoracic spine	0.04	0.02	0.02	—	4.9
Routine chest	0.14	0.06	0.08	—	7.8
Mediastinum	0.11	0.03	0.04	—	7.6
Routine abdomen	—	8.0	8.0	0.70	7.6
Liver	—	1.0	1.2	0.03	7.2
Pancreas	—	0.35	0.41	0.01	4.8
Kidneys	—	1.1	1.3	0.03	6.3
Adrenals	—	0.10	0.12	—	3.4
Lumbar spine	—	2.4	2.7	0.06	3.3
Routine pelvis	—	26	23	1.7	7.1

Multiply by 100 to convert mGy to mrad and mSv to mrem.

Adapted from NRPB 1992: Protection of the patient in x-ray computed tomography. Documents of the NRPB 3(4), 1992, with permission.

**TABLE 14.11.** Effective Doses from Computed Tomography

Examination	Mean Effective Dose, mSv	
	United Kingdom, 1989	Wales, 1994
Routine head	1.8	1.6
Posterior fossa	0.7	1.2
Pituitary	0.6	0.9
Internal auditory meatus	0.4	1.0
Facial bones	0.7	0.3
Orbits	0.6	0.8
Cervical spine	2.6	1.5
Thoracic spine	4.9	2.4
Lumbar spine	3.3	3.3
Chest	7.8	9.7
High resolution lung	—	1.9
Abdomen	7.6	12.0
Liver	7.2	10.3
Pancreas	4.8	7.4
Kidneys	6.3	9.1
Pelvis	7.1	9.8

Multiply by 100 to convert mSv to mrem.

Adapted from Shrimpton PC, Wall BF, Hart D: Diagnostic medical exposures in the UK. *Appl Radiat Isot* 50:261-269, 1999, with permission.

**TABLE 14.12.** Computed Tomography Dosimetry Parameters for Three Age Groups Undergoing Abdominal Examinations

Parameter	≤1Dy	11-18 y	>18 y
Section dose, mGy	23.7 ± 4.1	18.5 ± 3.3	15.7 ± 3.9
Energy imparted, mJ	72.1 ± 24.4	183.5 ± 44.8	234.7 ± 89.4
Effective dose, mSv	6.1 ± 1.4	4.4 ± 1.0	3.9 ± 1.1

Table data are mean ± SD.

Adapted from Ware DE, Huda W, Mergo PJ, Litwiler AL: Radiation effective doses to patients undergoing abdominal CT examinations. *Radiology* 210:645-650, 1999, with permission.

giography, which at about 10.6 mSv (1,060 mrem) is similar to that from an alternative nuclear-medicine study, but much more than a CT scan, and nearly two orders of magnitude more than from a plain skull x-ray.

Bone mineral densitometry is the subject of Table 14.14. There are wide variations for different techniques, but note that the units of effective doses are very small, in the microsievert range.

#### Collective Effective Dose

Next to be considered is the effect of diagnostic radiology on the population as a whole, rather than on the individual. The relevant quantity here is the collective effective dose, which is the product of the effective dose and the number of individuals exposed. It can give a very rough indication of the harm or detriment to an exposed population in terms of the number of radiation-induced cancers or heritable effects produced.

**TABLE 14.13.** Effective Doses from Cerebral Angiography

Procedure	Effective Dose, mSv
Cerebral angiography	10.6
Nuclear medicine:	about 10
Co <sup>67</sup> T tomography	2
Skull x-ray	0.15

Adapted from Feygelman VM, Huda W, Peters K: *AJR* 13:845-849, 1992, with permission.

TABLE 14.14. Representative Effective Doses from Bone Mineral Densitometry

Type of Measurement	Effective Dose, (iSv)	Comments
Dual energy x-ray absorptiometry	-2.5	Representative value for single PA scan
Single energy quantitative CT	-300	SPR + 3 CT slices @ 80 kVp
Dual energy quantitative CT	-1000	SPR + 3 CT slices @ 80 kVp + 3 CT slices @ 120 kVp
Radiographs	-100	Single (collimated) view (AP or lateral)

CT, computed tomography.

Adapted From Huda M, Morin RL: Brit J Radiol 69:422-425, 1996, with permission.

There are two values of the collective effective dose that are of interest in diagnostic radiology, that to the patient and that to healthcare workers.

First we consider the patient. Table 14.15 shows the collective effective dose from all diagnostic x-ray procedures performed in both doctors' offices and hospitals in the United States in 1980. The total amounts to 92,000 person-Sv (9.2 million person-rem). This estimate is out of date and much too low for the present time because of the greatly increased use of CT scans in the past two decades. On the other hand it may be too high, because the population receiving diagnostic x-rays is substantially skewed with regard to age distribution compared with the working population, for which the concept of effective

dose initially was developed. Over one half are older than 45 years of age, and a quarter are older than 64 years. It also has been claimed that half of all x-ray diagnostic procedures are performed on patients during a terminal illness within 18 months of their death; clearly, radiation-induced cancer and hereditary effects are largely irrelevant to this section of the population.

In any event, the collective effective dose in Table 14.15 may be too low or too high for various reasons, but it represents the best data available and is only a ballpark figure. Based on the International Committee on Radiological Protection (ICRP) estimates of 4% per sievert for fatal cancer induction and 0.6% per sievert for severe hereditary effects, this leads to the conclusion that 1 year's practice of diag-

TABLE 14.15. Collective Effective Dose from Diagnostic Medical X-Rays: United States, 1980

Examination Type	Effective Dose, mSv <sup>a</sup>	Thousands of Examinations	Collective Effective Dose, person-Sv <sup>b</sup>
Computed tomography (head and body)	1.11	3,300	3,660
Chest	0.08	64,000	5,120
Skull	0.22	8,200	1,800
Cervical spine	0.20	5,100	1,020
Biliary	1.89	3,400	6,430
Lumbar spine	1.27	12,900	16,400
Upper gastrointestinal	2.44	7,600	18,500
Abdomen (kidneys, ureters, bladder)	0.56	7,900	4,420
Barium enema	4.06	4,900	19,900
Intravenous pyelogram	1.58	4,200	6,640
Pelvis	0.44	0.64	3,010
Hip	0.83		
Extremities	0.01	45,000	450
Other	0.50	(8,400)	4,200
Rounded total			92,000

<sup>a</sup>1 mSv = 100 mrem.

<sup>b</sup>1 person-Sv = 100 man-rem.

Adapted from National Council on Radiation Protection and Measurements: Exposure of the US Population From Diagnostic Medical Radiation. Report No. 100. Bethesda, MD, NCRP, 1989, with permission.

nostic radiology results in the induction of about 3,680 cancer deaths in the individuals exposed and about 542 severe hereditary defects in future generations. This detriment must be balanced against the diagnostic benefit to the several hundred million individuals receiving the various procedures. Put another way, less than 0.004% of the examined population is potentially at risk, according to these estimates.

Some more recent data are available from the United Kingdom concerning CT scanning. A CT scanner with a full workload may result in an annual effective dose of about 22.5 person-Sv (2,250 person-rem). This may vary by a factor of up to 3 between different models from various manufacturers because of design differences. On the broad basis of such scanner workloads, however, it has been estimated that, in the late 1990s, CT scanning may have contributed around 40% of the collective effective dose to the population from radiologic procedures, although it represents only about 4% of the total number of procedures. This is a sobering thought.

In regard to collective doses to healthcare workers, in 1989 the NCRP published an extensive report on doses to medical staff compared with all radiation workers. Table 14.16 shows the estimated collective effective doses; Table 14.17 shows the breakdown for

the various environments in which x-rays are used for medical purposes; the bulk of the collective effective dose is about equally divided between hospitals and private practice. If this collective dose of 410 person-Sv for medical workers is multiplied by the JCRP risk estimate for radiation-induced cancer at a low dose rate of 4% per sievert, it leads to the conclusion that about 16 individuals in the U.S. healthcare industry will develop a fatal cancer as a consequence of each year's use of medical radiation.

### Estimating Risks at Low Doses

In this chapter, ICRP risk estimates for radiation-induced cancer and hereditary effects, combined with values of the collective effective dose, have been used to estimate the detriment caused by diagnostic radiology to patients and medical staff.

It is important to note that there is no direct evidence that small doses of radiation, similar to those used in diagnostic radiology, cause harmful effects in the persons who are exposed. Discussions in this chapter involve inferences and estimates of the biologic effects that might occur if a large number of persons are exposed to small doses of radiation, based on extrapolation from the known deleterious

TABLE 14.16. Collective Effective Doses to Radiation Workers

Occupational Category	Annual Collective Effective Dose, person-Sv
Industrial personnel (other than nuclear fuel cycle)	390
Nuclear power plant personnel	551
Department of Energy personnel	224
Uranium miners	112
Uranium mill and fuel fabrication personnel	6
Well loggers	30
US Public Health Service personnel	0.3
US Navy	51
Flight crews and attendants	165
Medical staff (non-Federal)	410
Government	120
Other workers	145
Education and transportation personnel	50
Rounded total	2,200

1 person-Sv = 100 man-rem

Adapted from National Council on Radiation Protection and Measurements: Exposures of the US Population from Occupational Radiation. Report No. 101: Bethesda, MD, NCRP, 1989, with permission.

TABLE 14.17. Summary of Mean Collective Equivalent Doses to Monitored Medical Workers

Sources of Occupational Exposures	Thousands of Workers	Collective Equivalent Dose, <sup>3</sup> person-Sv
Dentistry	259	60
Private medical practice	155	160
Hospital	126	170
Other <sup>6</sup>	44	20
Total	584	410

Collective equivalent doses are reported in the source of these data, but because the data were obtained from personnel monitors worn at waist level, the readings are assumed to represent total-body exposures; hence collective equivalent dose is identical to collective effective dose.

"Other" includes chiropractic medicine with 15,000, podiatry with 8,000, and veterinary medicine with 21,000 potentially exposed workers.

Adapted from National Council on Radiation Protection and Measurements: Exposures of the US Population from Occupational Radiation. Report No. 101: Bethesda, MD, NCRP, 1989, with permission.

effects observed if smaller number of persons (or animals) are exposed to much larger doses of radiation. All that can be done in the case of low doses of radiation is to make estimates, based on plausible assumptions, but these are estimates for which there is no direct evidence. They are not measurements or observations.

## INTERVENTIONAL RADIOLOGY AND CARDIOLOGY

The 1990s have witnessed a major increase in high-dose fluoroscopically guided interventional procedures in medicine. These procedures include cardiac radiofrequency ablation, coronary artery angioplasty and stent placement, neuroembolization, and transjugular intrahepatic portosystemic shunt placement. Such procedures tend to be lengthy and involve fluoroscopy of a single area of anatomy for a prolonged period of time—frequently for longer than 30 minutes and occasionally for over 1 hour. In addition, the need for multiple sequential sessions of treatment can occur. Because of the high skin doses that can be generated in the course of these interventions, some procedures have resulted in early or late skin reactions, including necrosis in some cases. In all cases of skin reactions, the doses are thought to have been high and the severity of some has required skin grafts or myocutaneous flaps for treatment. Procedures have evolved to include increasingly complex curative interventions that are asso-

ciated with higher radiation exposures to both patients and healthcare workers.

Angiography consists of inserting a catheter in and, using fluoroscopy, guiding it along, and injecting contrast material into, the vascular system. Stenosis or blockage of one or more of these vessels can lead to a myocardial infarction but can be visualized and treated with surgery or coronary angioplasty. Coronary angiography is a procedure in which cineangiography is used in angulated projections, which can expose the operator to a higher dose than if the x-ray equipment is used in the standard posteroanterior position.

Percutaneous transluminal coronary angioplasty is a therapeutic procedure to open blocked arteries by either inflating a small balloon inside the artery, compressing and fracturing the obstruction, or using rotating cutting blades to remove the obstruction. It often requires the deployment of stents, as well, to maintain vessel patency. During conventional coronary angioplasty, prolonged fluoroscopy in severely angulated positions increases the dose to the operator and the patient. For coronary angioplasty, the overall potential radiation exposure to the operators is medium to high. The usual arterial accesses are the femoral artery in the groin and the brachial artery in the shoulder. Large doses also are involved in the placement of transjugular intrahepatic portosystemic shunts.

The clinical application of electrophysiology in cardiology is to study the electrical conduction pathways of the heart. In the car-

diac catheterization laboratory, fluoroscopic control is used to position catheters in the heart to measure electric activity and to map electric conduction pathways. This technique is being used increasingly because abnormal conduction pathways, which may lead to life-threatening cardiac arrhythmias, can be controlled by ablation, now being performed using catheter-directed methods. Either a direct current or radiofrequency generator is used to produce a voltage of 20 or 30 V, which heats tissue to approximately 60°C for about 1 minute; this destroys the abnormal electric conduction pathways. These procedures usually require only posteroanterior fluoroscopy, although oblique or angulated views also are used. The successful treatment of some cardiac arrhythmias by radiofrequency ablation probably will increase the use of fluoroscopy for this type of therapy. Long fluoroscopic times and the occasional use of angulated views results in high radiation exposures to workers during this procedure.

In addition, a large number of nonvascular interventional procedures are performed such as the drainage of a blocked kidney or ablation of liver cancer.

### Patient Doses

Radiation doses received by patients from interventional radiology and cardiology are very much higher than from general diagnostic radiology, so much so that there is a risk of deterministic effects, such as early or late skin damage. This is not to say, however, that stochastic risks are absent. They are greater than for diagnostic procedures, because the doses are much higher. Because the patients are, in general, older and suffering from life-threatening medical conditions, however, the possibility of a radiation-induced cancer 10, 20, or 30 years down the road is largely academic. The immediate threat of deterministic effects, however, is very real and can affect quality of life in a serious way.

In view of the concerns raised by reports of adverse biologic effects, a number of attempts have been made to measure and document

doses received by patients undergoing diagnostic and interventional cardiac catheterization procedures. Patients have been reported to develop chronic radiodermatitis after multiple cardiac catheterizations for coronary angioplasties. In addition, several cases of radiation-induced severe skin damage have been reported to the FDA from fluoroscopically guided interventional procedures.

To put things into context, considering the hundreds of thousands of patients involved each year in the United States alone, problems seen in cardiac and neurologic intervention are exceedingly rare, but the potential is always there. Approximately 40 injuries were reported to the FDA in 1994. Although the entrance exposure limit for standard operation of a fluoroscope is 100 mGy/min (10 R/min), there is no limit on entrance exposure rate during any type of recorded fluoroscopy, such as cinefluorography or digital acquisitions. Consequently, dose rates of up to 500 mGy/min (50 R/min) and higher may be encountered during recorded interventional and cardiac catheterization studies. Several authors have estimated the entrance dose to the skin of the patient's back during cardiovascular procedures. Mean entrance doses for diagnostic catheterization vary from 0.7 to 2.2 Gy (70 to 200 rad). Difficult cases, and multiple procedures in the same patient, can readily lead to much higher doses that result in an erythema or more severe skin damage.

Studies have also been published on skin doses involved in patients who underwent arrhythmia ablation procedures. The average length of fluoroscopy was 46 minutes, corresponding to an entrance skin dose of about 1 Gy (100 rad), but a few percent of patients recorded a skin dose in excess of 3 Gy (300 rad). It should be noted that the radiation doses involved in a given procedure vary widely due to uncontrollable factors above and beyond the skill and experience of the operator, including the age, size and state of health of the patient.

Table 14.18 summarizes fluoroscopy time, cine time, and area exposure product (AEP), with units of Gy cm<sup>2</sup>, for diagnostic, inter-

**TABLE 14.18.** Fluoroscopy Times, Cine times, and Area-Exposure Products for Diagnostic, Interventional, and Combined Procedures

	Diagnostic (n = 173)	Interventional (n = 225)	Combined (n = 112)
Time, min			
Fluoroscopy	6.8 ± 6.4	19.9 ± 13.6	20.4 ± 10.5
Cine	0.78 ± 0.32	0.91 ± 0.6	1.18 ± 0.55
Area-exposure product Gy cm <sup>2</sup>			
Fluoroscopy	39 ± 46	101 ± 76	107 ± 65
Cine	70 ± 36	62 ± 33	92 ± 38
Total	108 ± 74	163 ± 95	198 ± 87
Cine runs	95 ± 33	136 ± 58	172 ± 59
Fluoroscopy <sup>a</sup>	32 ± 15	58 ± 14	52 ± 12

<sup>a</sup>Fluoroscopy expressed as a percentage of total area-exposure product

Adapted from Bakalyar DM, Castellani MD, Safian RD: Radiation exposure to patients undergoing diagnostic and interventional cardiac catheterization procedures. *Cathet Cardiovasc Diagn* 42:121-125, 1997, with permission.

vential, and combined procedures. It is not difficult to accept the idea that, above a threshold, deterministic effects such as damage to the skin depend in some way on the dose and the area exposed, but the exact form of the relationship is not obvious on radiobiologic grounds.

Table 14.19 is a much more detailed survey of the AEP values corresponding to a number of specific procedures. This survey also calculated effective doses, which are discussed in the next section. To put things into context, for a nominal irradiated area of 100 cm<sup>2</sup> at the skin surface, an AEP of 200 Gy cm<sup>2</sup> (20,000 rad cm<sup>2</sup>) would be required for a 2-Gy (200-rad) skin exposure, which is about the threshold for early transient erythema.

Several other conclusions were drawn from this and other studies. For example, radiation exposure was increased significantly in patients with a prior history of bypass surgery, because of the need for more cine runs to image the native vessels and bypass grafts, as well as more fluoroscopy time to cannulate the grafts, and in patients undergoing multi-vessel intervention. The entrance exposure rate may be in the region of 0.1 Gy/min (10 rad/min), and procedures may last from minutes to several hours.

Advances in spiral or helical CT scanning have led to the development of CT fluoroscopy (CTF). CTF presently is used as an imaging aid in invasive procedures, such as

accurate localization of a biopsy needle. Because the x-ray tube and detector system can rotate continually around the patient, spiral CT has made it possible to continually image the same cross-section of anatomy and have the reconstructed images displayed at many frames per second, demonstrating the temporal advancement of the invasive device. To manage the radiation at an acceptable level, the tube currents for CTF are much lower than those for routine CT scanning (about 10-20% that of normal). Even so, dose rates are substantive, and the potential for extremely high doses in a prolonged procedure exists. Doses to the skin of a patient after about 90 seconds of CTF range from about 0.2 to 0.5 Gy (20-50 rad). Prolonged CTF or CTF at high tube currents has the potential for serious injury to the patient. Doses to the physician performing the procedure are also substantive.

Because the maximum dose is to the skin, and because it is an external organ readily observable, it is not surprising that effects such as erythema or epilation are the most frequently reported. Radiation effects in the skin (Table 14.20) include the following:

- Early transient erythema may occur in a matter of hours following doses of more than 2 Gy (200 rad), because of changes in permeability of capillaries. The main wave of erythema peaks at 10 days to 2 weeks and requires a larger dose of about 6 Gy (600 rad).

**TABLE 14.19.** Mean Fluoroscopy Screening Times, Dose-Area Product Values

Interventional Procedure	Fluoroscopy Screening Time, min	Dose-Area Product, Gy cm <sup>2</sup>			Effective Dose, mSv
		Fluoroscopy	Radiography	Total	
<b>Diagnostic</b>					
Cerebral angiography	12.1	28.2	45.8	74.1	7.4
Carotid angiography	10.3	22.9	26.4	49.3	4.9
Upper extremity angiography	4.6	10.5	16.8	27.3	0.3
AV fistula angiography	2.3	4.6	12.6	17.2	0.2
Thoracic angiography	22.1	49.0	36.2	85.2	11.9
Nephrostography	4.0	12.4	2.2	14.7	2.4
Renal angiography	5.1	17.7	22.1	39.8	6.4
PTC	14.6	76.9	3.3	80.2	12.8
CT arterial portography	10.0	69.0	11.6	80.6	12.9
Hepatic angiography	12.1	74.9	61.0	136	21.7
Transjugular hepatic biopsy	6.8	30.8	3.4	34.1	5.5
Abdominal angiography	8.0	46.1	72.1	118	18.9
Femoral angiography	7.2	17.2	29.6	46.7	7.5
Lower extremity angiography	7.5	28.0	51.9	79.8	0.8
<b>Therapeutic</b>					
Cerebral embolization	34.1	43.1	61.4	105	10.5
AV fistula angioplasty	14.6	16.4	8.7	25.1	0.3
Thoracic therapeutic procedures	14.9	59.5	56.9	116	16.3
Biliary stent insertion/removal	7.1	40.5	2.6	43.1	6.9
TIPS	48.4	400	125	524	83.9
Nephrostomy	7.0	39.8	3.2	43.0	6.9
Renal angioplasty	14.0	57.0	28.1	85.2	13.6
Other abdominal therapeutic procedures (excluding hepatic and renal)	18.4	114	54.1	168	26.9

Adapted from McParland BJ: A study of patient radiation doses in interventional radiological procedures. Br J Radiol 71:175-185, 1998, with permission.

**TABLE 14.20.** Potential Effects of Fluoroscopic Exposures on the Reaction of the Skin

Effect	Approximate Threshold Dose, Gy	Time of Onset
Early transient erythema	2	2-24 h
Main erythema reaction	6	>1.5 wk
Temporary epilation	3	>3 wk
Permanent epilation	7	>3 wk
Dry desquamation	14	>4 wk
Moist desquamation	18	>4 wk
Secondary ulceration	24	>6 wk
Late erythema	15	>8-10 wk
Ischemic dermal necrosis	18	>10 wk
Dermal atrophy (1st phase)	10	>12 wk
Dermal atrophy (2nd phase)	10	>52 wk
Telangiectasis	10	>52 wk
Delayed necrosis	12?	>52 wk (related to trauma)
Skin cancer	Not known	>15 y

Adapted from Wagner LK, Archer BR: Minimizing Risks from Fluoroscopic X-rays, 2nd ed. Houston, TX, Partners in Radiation Management, 1998, with permission, and modified by Hopewell (personal communication).

- Dry desquamation, flaking sheets of corneum, much like a sunburn, may occur after single doses of more than 10 Gy (1000 rad) because of depopulation of clonogenic cells in the epidermis. Healing requires the repopulation of basal cells from surviving clonogens.
- Moist desquamation requires higher doses exceeding 15 Gy (1,500 rad) and also results from depopulation of clonogenic cells in the epidermis. Healing is caused by repopulation of surviving clonogens or migration of clonogens from the edges of the irradiated area. These effects may cause substantial discomfort, but provided they are not too severe, they heal and clear up as the population of basal cells recovers.
- Epilation, or hair loss, occurs if there is sufficient reduction in the replicative capacity of germinal cells or the matrix of the hair follicles. Temporary epilation may occur after doses of about 3 Gy (300 rad), with an onset at about 3 weeks and regrowth requiring 5 weeks or more. Epilation is permanent if the dose exceeds about 7 Gy (700 rad).
- Late effects in the skin include dermal atrophy, telangiectasia, and necrosis. These effects occur months to years after higher doses of radiation and are caused primarily by vascular damage to the dermis. Late effects also may develop after unusually severe early effects, in which case they are referred to as "consequential" late effects.

Table 14.20 is a compilation of the approximate dose levels and relative times at which these effects are observed following a single acute radiation exposure. The doses from fluoroscopically guided interventional procedures are so large that the potential effects of immediate concern are deterministic rather than stochastic.

### Effective Dose and Cancer

Although the entrance skin dose is the most relevant to the possible induction of acute deterministic effects, the effective dose is the relevant quantity for estimating the risks of sto-

chastic effects such as cancer. Effective dose is a function of the dose and the specific organs and tissues exposed and can be determined from mathematic models and measurements in an anthropomorphic phantom. Table 14.19 includes estimates of the effective dose for a wide range of procedures. Table 14.21 is more of a summary of mean doses, with less detail, of effective doses from arrhythmia ablation, coronary angiography, and coronary angioplasty. Quantitative estimates of the risk of cancer as a function of dose come from studies of the Japanese survivors of the atomic bombs, as described in Chapter 10, on radiation carcinogenesis. Converting these estimates to AEP values leads to a figure of 40 per billion exposed per  $\text{R cm}^2$ . The risk of cancer death, therefore, from a typical interventional procedure is of the order of 1 in 1,000.

Of course, these deleterious effects of radiation must be viewed in the context of the overall clinical situation. Most patients undergoing fluoroscopically guided interventional procedures are suffering from life-threatening conditions; they are likely to die unless something is done, and so the risk-benefit equation is heavily weighted by a substantial benefit balancing the undoubted risk.

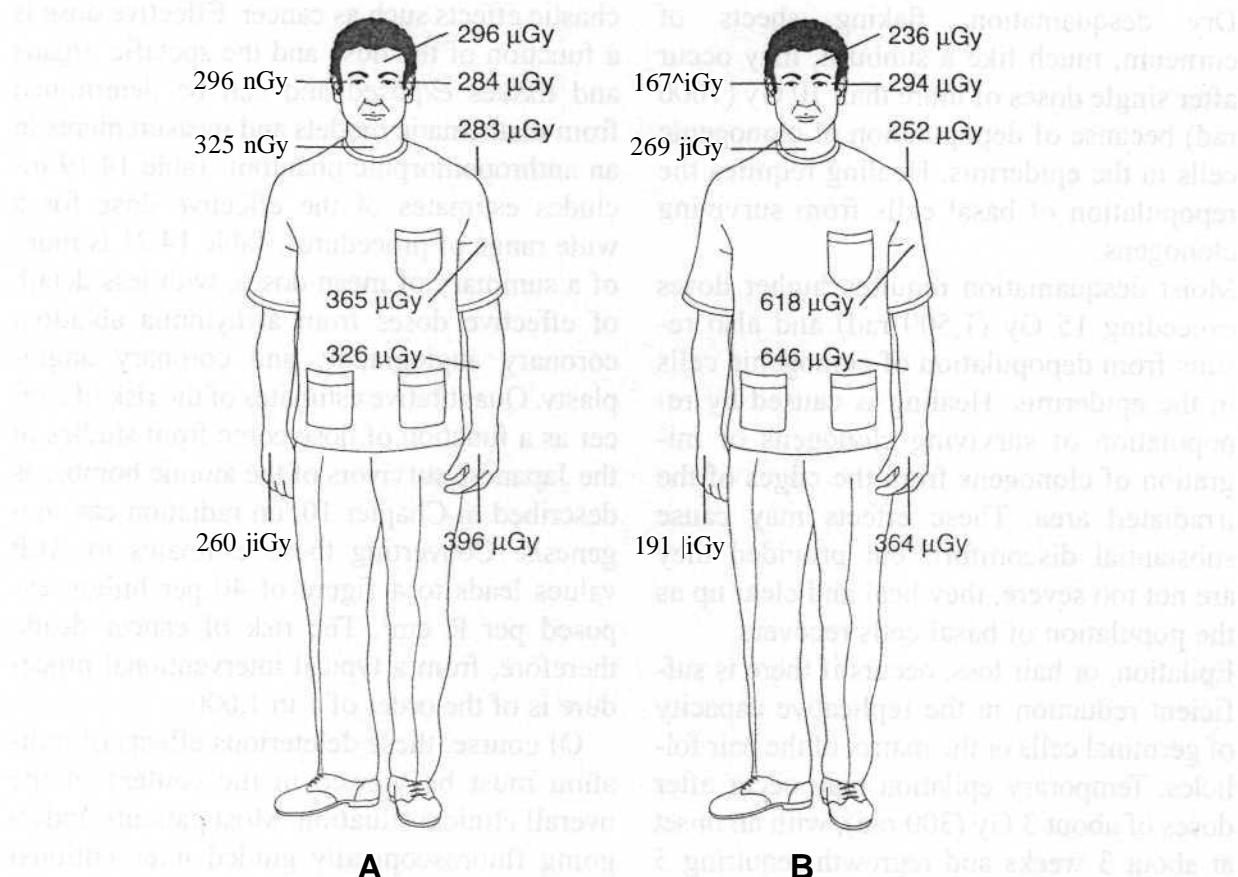
### Dose to Personnel

Physicians involved in cardiology, angiography, and fluoroscopically guided interventional work routinely receive radiation doses higher than any other staff in a medical facility, and comparable to doses received by workers in the nuclear industry (Fig. 14.6 and Table 14.22).

TABLE 14.21. *Effective doses to Patients from Radiological and Nuclear Medicine Procedures*

Procedure	Effective Dose, mSv
Arrhythmia ablation	17
Coronary angiography	12
Coronary angioplasty	22
Thallium-201 scan	21
Technetium-99 radionuclide ventriculogram	8

Adapted from Lindsay BD, Eichlin JO, Ambus HD, Cain ME: Am J Cardiol 70:218-223, 1992, with permission.



**Figure 14.6.** Graphic representation of the mean values of doses per procedure for a radiologist (A) and a cardiologist (B) engaged in an interventional procedure. The figures are the mean of measurements taken during more than eighty procedures. (Adapted from Vano E, Gonzalez L, Guiberalde E, Fernandez JM, Ten JL: Br J Radiol 71:954-960, 1998, with permission.)

This is principally because of the use of fluoroscopy. CTF poses a new and additional risk.

## NUCLEAR MEDICINE

### Historical Perspective

The first person to suggest using radioactive isotopes to label compounds in biology and medicine was the Hungarian chemist Hevesy, whose work, beginning before World War II, earned him a Nobel Prize in 1943 (Fig. 14.7). The concept of using radioactive tracers in medicine could not be exploited until the means to produce artificial isotopes were readily available. The cyclotron was invented by Ernest Lawrence in the 1930s and has been used to produce short-lived isotopes and positron emitters (Fig. 14.8). Nuclear reactors

were developed during World War II and are used to produce most medically used radioactive isotopes, all of which are electron or y-ray emitters.

For these reasons nuclear medicine was a late starter compared with radiation therapy and x-ray diagnosis. Radiopharmaceuticals of adequate quality and consistency were not available until 1946, but since then nuclear medicine has grown into a specialty in its own right and was one of the most rapidly growing areas of medicine until slowed by the advent of CT scanning and magnetic resonance imaging. A broad array of pharmaceuticals, coupled with the development of sophisticated hardware, has made possible a widening diversity of applications. PET scanning has opened up a whole new area of rapid growth that is discussed subsequently in this chapter.

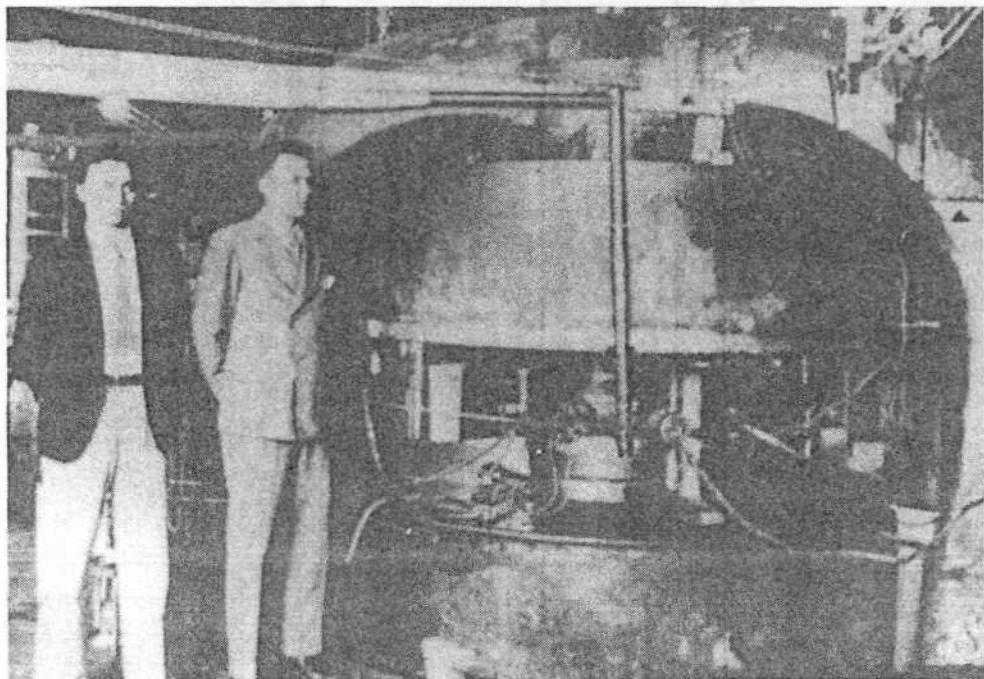
TABLE 14.22. *Estimated Dose to Staff During Typical Cardiac Studies*

Category of Staff	One Catheterization, mSv				One Angioplasty, mSv				One Pacemaker Implant (No Cine), mSv			
	Weighted Surface Dose, No Apron	Weighted Surface Dose with Apron	Hands	Eyes	Weighted Surface Dose, No Apron	Weighted Surface Dose with Apron	Hands	Eyes	Weighted Surface Dose, No Apron	Weighted Surface Dose with Apron	Hands	Eyes
Cardiologist	1.6	0.09	2.1	0.6	3.1	0.2	4.2	1.0	0.14	0.01	0.2	0.05
Cardiologist who stands back during cine	0.3	0.01	0.3	0.2	1.5	0.1	1.9	0.7				
Technologist	0.08	<0.01	0.09	0.02	0.2	0.01	0.2	0.05	0.01	<0.01	0.01	<0.01
Technologist who stands back during cine	0.04	<0.01		0.04	0.01	0.1	0.01	0.1	0.03			
Nurse or anesthetist	0.3	0.02	0.4	0.2	0.8	0.06	0.9	0.5	0.04	<0.01	0.04	0.03

Adapted from National Council on Radiation Protection and Measurements: Implementation of the Principle of As Low As Reasonably Achievable (ALARA) for Medical and Dental Personnel. Report No.107. Bethesda, MD, NRCP, 1990, with permission.



**Figure 14.7.** The great Hungarian chemist Hevesy, whose work beginning before World War II earned him a Nobel Prize in 1943. He was the first to conceive of using radioactive isotopes to label compounds for biology and medicine. (Courtesy of the University of California Lawrence Berkeley Laboratory.)



**Figure 14.8.** The concept of using radioactive isotopes as tracers in medicine was not fully explored until the invention of the cyclotron in 1931. Its inventor, Ernest O. Lawrence, is seen here (right) with his second cyclotron in 1934. Many short-lived isotopes are made with a device of this sort. (Courtesy of the University of California Lawrence Berkeley Laboratory.)

It is estimated that at the present time there are more than 10,000 physicians in the United States licensed to administer radiopharmaceuticals to patients for diagnostic and therapeutic purposes. In 1989 the NCRP estimated that about 100 million procedures using radioactive materials are performed each year in the United States for diagnostic and therapeutic medical purposes. Approximately 10% of these procedures involve administration of radioactive pharmaceuticals directly to patients for diagnostic or therapeutic procedures. The remaining 90% are radioimmunoassay procedures that involve the use of small amounts of radioactivity in analysis of patient urine, blood, and so forth.

Although there are over 150 diagnostic and therapeutic nuclear-medicine procedures involving the administration of radiopharmaceuticals to patients, only 10 *in vivo* diagnostic procedures comprise over 90% of all such

procedures performed in a typical nuclear-medicine clinic, and only one therapeutic procedure constitutes the bulk of all nuclear-medicine treatments (Table 14.23). The distribution of studies has shifted significantly over the years, reflecting two simultaneously occurring trends. Radiologic imaging has improved significantly with the advent and application of high-contrast, high-resolution modalities (CT scanning, ultrasound, magnetic resonance imaging, and digital subtraction angiography) for anatomic definition, thereby supplanting the poorer-resolution nuclear-medicine techniques in the detection and definition of pathologic anatomy. On the other hand, pathophysiologically oriented nuclear-medicine studies have made significant progress with the availability of newer radiopharmaceuticals (*e.g.*, myocardial perfusion agents, regional cerebral blood flow agents), instrumentation (*e.g.*, single photon emission

**TABLE 14.23.** *Relative Frequency of Nuclear Medicine Procedures (1991), Typical Activities Administered, and Typical Dose*

Procedure	Relative Frequency of Procedure, %	Radiopharmaceutical	Activity Administered per Procedure, MBq	Typical Dose to Patient, mGy
Diagnostic				
Bone	20.6	$^{99m}\text{Tc}$ medronate or oxidronate	740	1.3
Gastric emptying	4.6	$^{99m}\text{Tc}$ sulfur colloid	40	0.2
Heart	11.8	$^{99m}\text{Tc}$ red cells	110	4.5
Equilibrium radiocardiography		$^{201}\text{TI}$ thallous chloride	110	6.3
Myocardial perfusion	17.9	$^{99m}\text{Tc}$ sestamibi $^{99m}\text{Tc}$ teboroxime	<b>1,110</b> 1,850	5.0 8.3
Hepatobiliary	2.9	$^{99m}\text{Tc}$ disofenin $^{131}\text{I}$ iodohippurate	300 15	<b>1.3</b> 0.4
Kidney	9.6	$^{99m}\text{Tc}$ penetate $^{99m}\text{Tc}$ mertiatide	370 370	0.6 0.7
Lung	8.2	$^{99m}\text{Tc}$ macro-aggregated albumin $^{133}\text{Xe}$ gas	110 370	0.5 0.14
Perfusion				
Ventilation	7.3	$^{99m}\text{Tc}$ penetate aerosol	740	1.6
Thyroid (25% uptake of iodine)	5.6	$^{123}\text{I}$ Na iodide $^{131}\text{I}$ Na iodide	15 4	0.4 0.7
Tumor/infection	3.8	$^{99m}\text{Tc}$ pertechnetate	185	0.7
Other	5.7	$^{67}\text{Ga}$ citrate	190	13.0
Therapeutic				
Hyperthyroidism	1.8	$^{131}\text{I}$ Na iodide	740	—
Thyroid cancer	0.2	$^{131}\text{I}$ Na iodide	3,700	—

Based on National Council on Radiation Protection and Measurements: Sources and Magnitude of Occupational and Public Exposures from Nuclear Medicine Procedures. Report No 124. Bethesda, MD, NCRP, 1990.

CT), and computers and software (*e.g.*, renal function evaluation).

### Principles in Nuclear Medicine

The use of radiopharmaceuticals for diagnosis or therapy is based on the accumulation or concentration of the isotope in the organ of interest, which is referred to as the *target organ*. A radiopharmaceutical may have an affinity for a certain organ that is not necessarily the organ of interest, in which case this organ is termed the critical organ. Often the dose to a critical organ limits the amount of radioisotope that may be administered. The risk to which the patient is subjected is clearly a function of the doses received in all organs and must be balanced against the expected advantages and benefits rendered by the procedure. The calculation of the dose absorbed from a radiopharmaceutical can represent a tricky problem, because it may vary with a number of factors. These factors include the following:

- 1: The distribution of the radionuclide within the body and its uptake in certain critical organs
2. *Inhomogeneous* distribution of the nuclide even within the critical organ
3. The biologic half-life of the nuclide, which may vary with the patient's age and be modified by disease or pathologic conditions

The use of radionuclides in medicine is rather sharply divided into diagnostic and therapeutic procedures. There may be a thousand-fold difference in the amounts of radioactive material used and therefore in the doses absorbed, depending on whether a given isotope is used to aid diagnosis or for therapy. Consequently, therapeutic uses are discussed under a separate heading.

In general, there are three doses that are of interest after the administration of a given amount of a radiopharmaceutical:

1. The total-body dose, because this largely determines the risk of leukemia and cancer.
2. The gonadal dose, because this is a measure of heritable (genetic) effects.
3. The dose to the critical organ, because this may be many times larger than the total-body dose, and it is known that certain tissues are particularly susceptible to radiation-induced cancer.

The risk of cancer and heritable effect is related to the effective dose.

### Dose, Effective Dose, and Collective Effective Dose

Effective doses resulting from a few common nuclear medicine procedures are listed in Table 14.24; gonad doses characteristic of selected representative procedures are shown in Table 14.25. Such tables have been rendered largely obsolete by the exhaustive calculations of both organ doses and effective doses for every known radionuclide and procedure by the Oak Ridge Institute for Science and Education. The doses are calculated using the MIRD (Medical Internal Radiation Dose) technique as implemented in the MIRDOSE 3 computer code developed by the Radiation Internal Dose Information Center at the Oak Ridge Institute. Dose estimates are available for adults, for children, and for embryos or fetuses at various stages of gestation. The dose to the uterus may be used to estimate the dose to the developing embryo or fetus up to about 6 weeks of gestation.

To obtain electronic copies of documents containing these dose estimates, log on via anonymous FTP- to [ftp.orau.gov](ftp://ftp.orau.gov), using the word *anonymous* as the username and your e-mail address as the password. Once logged in, type "cd doses" and copy any of these documents that are of interest to you. The file *index.dos* contains a description of which files contain which information.

Alternatively, the data may be obtained by contacting Radiation Internal Dose Information Center, Oak Ridge Institute for Science

**TABLE 14.24.** Maximum Usual Activity Per Test Recommended in the United Kingdom and Corresponding Effective Dose for Some Common Diagnostic Nuclear Medicine Procedures

Procedure	Radiopharmaceutical	Maximum Usual Activity per Test, MBq	Effective dose, mSv
Bone scan	$^{99m}$ Tc phosphate compounds	600	3.5
Renal scan	$^{99m}$ Tc DMSA	80	0.7
Renal scan	$^{99m}$ Tc DTPA	300	1.6
Dynamic cardiac scan	$^{99m}$ Tc erythrocytes	800	5.3
Biliary scan	$^{99m}$ Tc IDA	150	2.3
Brain scan	$^{99m}$ Tc HMPAO	500	4.7
Abscess imaging	$^{99m}$ Tc leukocytes	200	2.2
Lung perfusion scan	$^{99m}$ Tc MAA	100	1.1
Renal scan	$^{99m}$ Tc MAG3	100	0.7
Myocardial imaging	$^{99m}$ Tc MIBI	400	3.4
Thyroid scan	$^{99m}$ Tc pertechnetate	80	1.0
Tumor/abscess imaging	$^{67}$ Ga citrate	150	16.5
Thrombus imaging	$^{111}$ In leukocytes	20	7.2
Thyroid scan (35% uptake)	$^{123}$ I iodide	20	4.4
Tumor imaging	$^{123}$ I MIBG	400	5.6
Thyroid metastase (0% uptake)	$^{131}$ I iodide	400	24
Myocardial imaging	$^{201}$ Tl chloride	80	18

Adapted from Shrimpton PC, Wall BF, Hart D: Diagnostic medical exposures in the UK. *Appl Radiat Isot* 50:261-269, 1999, with permission.

**TABLE 14.25.** Administered Activity and Gonadal Doses

Examination Type	Estimated Administered Activity per Examination <sup>a</sup>	Gonadal Dose for Each Radiopharmaceutical, mGy <sup>b</sup>		Gonadal Dose, Weighted Average, mGy <sup>b</sup>	
		Male	Female	Male	Female
Brain	740 MBq $^{99m}$ Tc DTPA (50) 740 MBq $^{99m}$ Tc O <sub>4</sub> (50)	2.2 1.5	4.4 4.4	1.9	4.4
Hepatobiliary	185 MBq $^{99m}$ Tc iminodiacetic acid (IDA) (10) 185 MBq $^{99m}$ Tc sulfur colloid (90)	0.2 0.2	1.7 0.4	0.2	0.5
Bone	740 MBq $^{99m}$ Tc phosphate	3.7	4.4	3.7	4.4
Respiratory				0.3	0.3
Perfusion	185 MBq $^{99m}$ Tc macroaggregated albumin (MAA) (66)	0.4	0.4		
Ventilation	370 MBq $^{133}$ Xe gas (34)	0.1	0.1		
Thyroid	185 MBq $^{99m}$ Tc O <sub>4</sub> (80) 3.7MBq $^{131}$ I (10) 11.1 MBq $^{123}$ I (10)	0.4 <0.1 <0.1	1.1 0.1 0.1	0.3	0.9
Renal	740 MBq $^{99m}$ Tc DTPA (60) 9.25 MBq $^{131}$ I hippuran (40)	2.2 <0.1	4.4 <0.1	1.3	2.7
Abscess/tumor	111 MBq $^{67}$ Ga citrate	7.2	8.4	7.2	8.4
Cardiovascular	740 MBq $^{99m}$ Tc labeled red blood cells (40) 111 MBq $^{201}$ Tl chloride (40) 740 MBq $^{99m}$ Tc phosphate (20)	0.2 45.5 3.2	0.8 11.1 4.4	18.9	5.7

<sup>a</sup>Number in parentheses is the estimated percent of examination type with a particular radiopharmaceutical.

<sup>b</sup>1 mGy = 100 mrad.

Adapted from National Council on Radiation Protection and Measurements: Exposure of the US Population From Diagnostic Medical Radiation Report No. 100. Bethesda, MD, NCRP, 1984, with permission.

and Education, P.O. Box 117 MS 51, Oak Ridge, TN 37831-0117, phone (423) 576-3448, fax (423) 576-8673.

Although the figures in Tables 14.23 and 14.24 must be regarded as purely representative, they are nevertheless instructive. For example, the effective dose for a typical nuclear medicine procedure, such as a bone scan using technetium-99m phosphate compounds, is about 3.5 mSv. Using the risk estimate of 4% per sievert for radiation-induced cancer, derived in the chapter on radiation carcinogenesis (Chapter 10), this leads to a risk associated with a bone scan of about 1.4 in 10,000. Alternatively, this is about the same as 1 year of exposure to natural radiation.

The other figure of interest is the collective effective dose, which is a measure of the impact of nuclear medicine on the entire U.S. population. The collective effective dose is the summed product of effective dose and the number of individuals exposed (see Chapter 15 for definitions). There have been no recent surveys of collective doses from nuclear medicine, but Table 14.26 is reproduced from NCRP Report 100 published in 1989. The biggest contributors to the collective effective dose are brain, bone, thyroid, and cardiovascular procedures. The age-weighted collective effective dose for nuclear medicine is reduced by an even larger fac-

tor than for diagnostic x-rays, to account for the fact that the patient population is skewed even more towards older individuals who are less likely to be actively reproductive and who are less susceptible to cancer induction. Consequently, hereditary effects are of little importance, and even the risk of radiation-induced cancer becomes moot if the patients do not live long enough for those late effects to be manifest. The age-weighted collective effective dose for nuclear medicine is estimated to be about 13,500 person-Sv (Table 14.26). Based on this figure, the practice of nuclear medicine in the United States for 1 year may result in 540 radiation-induced fatal cancers and 108 serious hereditary effects. Stated another way, less than 0.006% of those examined might be affected. This calculated possible risk must be weighted against the benefit that about 10 million patients receive from diagnosis of their medical conditions.

### Positron Emission Tomographic Studies

One exciting development in nuclear medicine in recent years involves the use of PET scanning. The important and unique feature of PET studies is that they document physiologic abnormalities, or changes in metabolism, rather than simply alterations in anatomy.

**TABLE 14.26. Comparison of Collective Effective Dose Versus Age-Weighted Collective Dose for US Nuclear Medicine Procedures in 1982**

Examination	Effective Dose, mSv	Examinations, $\times 10^3$	Collective Effective Dose, person-Sv	Age-weighted Collective Dose, person-Sv <sup>a</sup>
Brain	6.5	813	5,300	2,200
Hepatobiliary	3.7	180	700	300
Liver	2.4	1,424	3,400	1,300
Bone	4.4	1,811	8,000	2,900
Pulmonary	1.5	1,203	1,800	800
Thyroid	7.5	530	4,000	2,400
Renal	3.1	236	700	400
Tumor	12.2	121	1,500	600
Cardiovascular	7.1	961	6,800	2,600
Total			32,100	13,500
Per caput			140 $\mu$ Sv (14 mrem)	59 $\mu$ Sv (5.9 mrem)

<sup>a</sup>1 Sv = 100 rem.

Adapted from National Council on Radiation Protection and Measurements: Exposure of the US Population From Diagnostic Radiation. Report No. 100. Bethesda, MD, NCRP, 1989, with permission.

The principle of PET imaging is that the scanner locates the tracer by detecting the collinear pairs of 0.511-MeV photons emitted if a positron annihilates after uniting with an electron. A positron is a particle with the same mass and magnitude of charge as an electron, except that the charge is positive. A positron cannot exist at rest, because if it has lost all its kinetic energy, it is electrostatically attracted to an electron with which it annihilates to produce two antiparallel 0.511-MeV photons. Radionuclides that emit positrons have excesses of protons in their nuclei and are produced by bombarding stable elements in a cyclotron. Positron emitters do not occur in nature.

Examples of radionuclides used for PET imaging include oxygen-15, carbon-11, and fluorine-18; these radionuclides have short half-lives of 2, 20, and 110 minutes, respectively, so that the PET facility is frequently close to the cyclotron.

Brain imagers showing the physiologic and functional distribution of positron tracers are currently in use at about 200 PET imaging centers around the world. The most commonly administered positron-emitting radionuclide is fluorine-18, which is used for the production of [<sup>18</sup>F]-2-deoxy-2-fluoro-D-glucose, usually referred to as *FDG*. This material is used in routine clinical care. Most of the other positron-emitting radionuclides are

used for the production of experimental compounds used in research studies. For example, oxygen-15 is used to label water for blood-flow studies. The doses delivered to patients in PET studies are quite low because of the rapid decay of the radionuclides, even though the administered amounts are high to allow rapid and detailed imaging.

Table 14.27 shows organ doses as well as effective doses for studies with [<sup>18</sup>F]FDG and for [<sup>15</sup>O]<sub>2</sub>O. Much more detailed dosimetry is available through the internet from the Oak Ridge Institute for Science and Education. For procedures using [<sup>18</sup>F]FDG, the only agent used commonly in routine clinical care, the bladder wall receives the highest dose; the heart, brain, and kidney also receive relatively high absorbed doses. The ICRP, in its publication 53, estimated the effective dose from this procedure to be about  $2.7 \times 10^{-2}$  mSv/MBq, which is in good agreement with the Oak Ridge calculation. A typical administered dose may be 370 MBq (10 mCi), so that the effective dose would be about 11 mSv (1.1 rem). This is equal to about 4 months of natural background radiation. The associated risk of fatal radiation induced cancer might be about 0.04%, or 4 cases per 10,000.

PET technologists often have higher radiation exposure than other workers in nuclear medicine. Pharmacists at cyclotron facilities have even higher exposures, especially to

TABLE 14.27. Organ Doses and Effective Dose for Position Emission Tomography Compounds

	F-18FDG		O-15 H <sub>2</sub> O	
	mGy/MBq $\times 10^{-2}$	rad/mCi $\times 10^{-2}$	mGy/MBq $\times 10^{-3}$	rad/mCi $\times 10^{-3}$
Brain	1.9	7.0	1.3	4.9
Heart wall	6.0	22.0	2.2	8.2
Kidneys	2.0	7.4	1.9	7.2
Ovaries	1.7	6.3	0.36	1.3
Red marrow	1.3	4.8	0.90	3.3
Spleen	3.7	14.0	1.6	5.8
Testes	1.3	4.8	0.67	2.5
Thyroid	1.0	3.9	1.7	6.3
Bladder wall	19.0	70	0.22	0.81
	mSv/MBq $\times 10^{-2}$	rem/mCi $\times 10^{-2}$	mSv/MBq $\times 10^{-3}$	rem/mCi $\times 10^{-3}$
Effective dose	3.0	11.0	1.1	4.2

Data from the Oak Ridge Institute for Science and Education (ORISE).

their hands. This is a function of two factors. First, the relatively high energy, and therefore penetrating nature, of the photons emitted by the radionuclides used (0.511 MeV). Second, because of the short half-lives of the commonly used positron-emitting radionuclides, large initial activities must be prepared in order that a sufficient amount is left by the time the patient is imaged. This is especially true for oxygen-15, which has a half-life of only 2 minutes. The ultimate role of metabolic radiopharmaceuticals in clinical practice is yet to be determined. These agents, however, are of significant research interest in an increasing number of centers that have acquired PET capabilities.

### The Therapeutic Use of Radionuclides

Radioactive iodine-131 is widely used for the treatment of hyperthyroidism and thyroid cancer. Because this radionuclide first became generally available in 1946, there has been a gradual shift in the selection of definitive therapy for hyperthyroidism. In the late 1940s and early 1950s, iodine-131 was reserved for patients deemed to be poor surgical risks. By the 1960s the situation had been completely reversed. Radioactive iodine had become the preferred modality, with thyroidectomy being reserved for the young and for those patients whose clinical evaluation suggested the desirability of surgery to allow an examination of the gland. As a rough estimate, 250,000 patients have now received at least one therapeutic dose of radioactive iodine. Hyperthyroidism is probably the major benign disease for which radiation is the treatment of choice.

A therapeutic treatment with iodine-131 involves an absorbed dose to the thyroid gland that varies with the person and is very nonuniform within the tissue itself but is on the order of many tens of grays (thousands of rads). In addition, there is a total-body dose of typically 50 to 150 mGy (5-15 rad), which results from the isotope circulating in the blood. Because radiation is known to be a potent carcinogen, the possibility of the production of

leukemia after iodine-131 therapy has been appreciated from the outset and has been looked for carefully. There is also the risk of thyroid cancer, because the isotope is concentrated in the thyroid and a large dose is delivered there. These are discussed in turn.

There is no question that thyroid cancer can be induced by external x-irradiation, though most of the data refer to children. By contrast, no excess of thyroid cancer has been observed in adults after the iodine-131 treatment of hyperthyroidism. The experience of the Chernobyl accident indicates that, although children are very sensitive to the induction of thyroid cancer by radioiodine, adults are quite resistant.

Because of isolated reports of patients who developed leukemia after treatment with iodine-131 for hyperthyroidism, the Cooperative Thyrotoxicosis Therapy Follow-up Study was initiated in 1961 under the sponsorship of the U.S. Bureau of Radiological Health. The study included 36,000 patients in 26 medical centers in the United States who were treated for hyperthyroidism either by radioiodine or surgery. The mean bone-marrow doses from the iodine-131 treatments were in the range of 70 to 150 mGy (7-15 rad). The data failed to reveal a statistically significant excess of leukemia from the radioiodine treatment *per se*. It is of interest to note that the age-adjusted death rate from leukemia in both treatment groups was one and a half times higher than expected on the basis of figures for the general population, from which it was concluded that patients with hyperthyroidism have an enhanced risk of leukemia, regardless of the way in which they are treated.

Pregnancy is, of course, a contraindication to the treatment of hyperthyroidism with iodine-131. Treatment of fertile women should be preceded by the taking of a careful history and a pregnancy test. Treatment should be delayed, if possible, to eliminate the potential effects during pregnancy.

In the treatment of thyroid cancer many hundreds of millicuries of iodine-131 may be given, which results in a total-body dose sufficient to cause severe depression of the bone

marrow. For example,  $3.7 \times 10^3$  MBq (100 mCi) of iodine-131 delivers 0.5 to 1 Gy (50-100 rad) to the hematopoietic tissue. The effect of such doses on the circulating blood elements is similar to the effect of total-body exposure to external radiation. Ultimately, bone-marrow depression may limit the treatment. Because the treatment of thyroid cancer involves the use of such large and repeated quantities of iodine-131, with the attendant total-body doses, it is not surprising that a few cases of myeloid leukemia have been reported in patients who have received this form of therapy. This is the risk entailed whenever therapeutic doses of radiation are involved; it is usually acceptable because of the serious and malignant character of the disease under treatment.

### MEDICAL IRRADIATION OF CHILDREN AND PREGNANT WOMEN

#### Irradiation of Children

The hazards associated with medical radiation in children are basically the same as in adults—namely, cancer and heritable effects (mutations)—except for the possibility that the risks associated with a given absorbed dose of radiation are higher because of an increased sensitivity in younger persons. There is good evidence for this. The Japanese survivors of the atomic-bomb attacks represent the most carefully studied human population exposed to radiation. There is a marked decrease in sensitivity to radiation-induced malignancies with increasing age. The effect is most dramatic for breast cancer, in which case young girls and teenagers are so much more radiosensitive than older adults (Chapter 10). The same appears to be true of thyroid cancer, for which the incidence per unit dose is much higher in childhood. The most reliable estimates for thyroid cancer come from children irradiated externally with x-rays for a supposedly enlarged thymus, and from children irradiated in the course of the treatment of tinea capitis. In both cases the thyroid receives a

dose of radiation (as little as 60 mGy or 6 rad in the children with tinea capitis), and within 20 years an elevated incidence of thyroid cancer is evident. The overall lifetime risk for children irradiated before the age of 10 years is perhaps three times higher than for individuals irradiated as adults. The importance of age is even more obvious in the case of incorporated radioactive iodine. The Chernobyl study shows clearly that children ingesting radioactive iodine are very sensitive to the induction of both benign and malignant thyroid tumors, whereas adults appear to be relatively resistant.

Concern for possible heritable effects induced by radiation is likewise greater in children, because they have their entire reproductive lives ahead of them. For many adult patients who are past their reproductive years, the risk of heritable effects is clearly of no concern.

In pediatric radiology or pediatric nuclear medicine, the general principle is that radiation exposures should be kept to the lowest practical level. In each case the expected benefit should exceed the risk clearly. The dominant concern is the burgeoning use of CT imaging in pediatric radiology, because the doses involved are substantial and the effective doses for a given procedure are even larger in children than in adults, because critical organs are closer together (Table 14.12). Physicians and patients alike are much more cautious about nuclear-medicine procedures in children than about diagnostic x-rays, even if the dose levels may be similar.

The implication of the review of biologic effects on humans (Chapters 10 and 11) is that any amount of radiation, no matter how small, has a deleterious effect. This conclusion is based on the assumption of a linear, non-threshold, dose-effect model that has been adopted by most standard-setting bodies as the most conservative basis for risk estimates. This philosophy requires that the physician have some reasonable indications that the potential gain for the patient from the use of a procedure in nuclear medicine exceeds the risks. The demand for nonessential repetitive

examinations and the use of children in poorly planned "research" studies are examples of nonproductive uses of radiation.

### Irradiation of Pregnant Women

The risks involved in exposure to radiation of the embryo or fetus are discussed in detail in Chapter 12. They may be summarized as follows:

1. For the first 2 weeks following conception (*i.e.*, during preimplantation), the most significant effect of radiation may be to kill the embryo, leading to resorption. There is also a risk of carcinogenesis.
2. Between 2 and 8 weeks postconception, the risks include congenital malformation and small head size, as well as carcinogenesis.
3. Between 8 to 15 weeks, and to a lesser extent 15 to 25 weeks, the risks include mental retardation as well as small head size and carcinogenesis.
4. Beyond 25 weeks, the only risk of externally delivered diagnostic radiation is carcinogenesis, which is much reduced compared with the risk during the first trimester.

Radiation-induced carcinogenesis is considered to be a stochastic effect; that is, there is no threshold and the risk increases with dose.

One obstetric examination involving a few films may increase the relative risk of leukemia and childhood cancer by about 40%; however, because malignancies are relatively rare in children, the absolute risk is small. The other serious effects, such as mental retardation and congenital malformations, are considered to be deterministic; that is, there is a threshold of about 0.1 to 0.2 Gy (10-20 rad).

It is against the background of these possible risks that the irradiation of the pregnant or potentially pregnant woman must be considered.

Some radiologists instruct their technologists to ask all female patients about possible

pregnancy before they have abdominal or pelvic radiographic examinations. This would appear to be prudent and expedient. Indeed, if time permits, a pregnancy test might be desirable. If a woman requires an emergency radiologic examination, however, there should be no hesitation to do the study. The health of the woman is of primary importance, and if serious injury or illness is suspected, this takes priority in determining the need for a study. The risk to a possible conceptus must be weighed against the risks of not performing the examination.

The NCRP (in 1977) recommended that "if, in the best judgment of the attending physician, a diagnostic examination or nuclear medicine procedure at that time is deemed advisable to the medical well-being of the patient, it should be carried out without delay, with special efforts being made, however, to minimize the dose received by the lower abdomen (uterus)."

In the case of nonemergency examinations, it sometimes may be prudent to consider delaying the proposed procedure.

The physician contemplating the delay of a study on a woman early in pregnancy should consider the consequences in view of the possibility that the diagnostic examination might become necessary later in the pregnancy, when the risks are much greater. For example, during the first 2 weeks postconception, the risks are possible carcinogenesis and resorption of the embryo. If the study is delayed, however, but becomes essential during the 8th through the 15th week, the risks include small head size and carcinogenesis; in addition, this is the peak of sensitivity to mental retardation. Delay compounds the problem.

Conversely, if the patient is already in this peak period of radiosensitivity if a procedure is contemplated, then a delay until after the 25th week would be an advantage, because radiation risks during this period **may** be at their smallest and involve only the slight risk of carcinogenesis. The effects of radionuclides on the developing embryo or fetus have not been studied as extensively as the consequences of externally administered x-rays.

TABLE 14.28. Thyroidal F<sup>131</sup>Iodine Dose to the Fetus

Gestation Period	Fetal/Maternal Ratio (Thyroid Gland)	Dose to Fetal Thyroid, rad/ $\mu$ Ci <sup>a</sup>
10-12 weeks	—	0.001 (precursors)
12-13 weeks	1.2	0.7
Second trimester	1.8	6
Third trimester	7.5	—
Birth imminent	—	8

<sup>a</sup>Rad/ $\mu$ Ci of <sup>131</sup>I ingested by mother.

Courtesy of Dr. J. Kereikes, unpublished data, 1984.

The biologic effects may depend on many factors, including the chemical form of the isotope, the type and energy of the radiation emitted, whether the compounds containing the radioactivity cross the placenta, and whether they tend to be concentrated in specific target organs.

The metabolism of the radiopharmaceutical may cause high concentrations of the radionuclide in organs of a conceptus if the material crosses the placenta. This may result in dysfunctional fetal organs. The classic example of this effect involves the use of iodine-131 after the eighth week postconception. Prior to this time there is no preferential uptake of iodine in the thyroid. After this time, iodine concentrates in the fetal thyroid in amounts considerably greater than those in the maternal thyroid (Table 14.28). A number

TABLE 14.29. Dose Estimate to Embryo from Radiopharmaceuticals

Radiopharmaceutical	Embryo Dose, rad/mCi administered
<sup>67</sup> Ge-citrate	0.25
<sup>5</sup> Se-methionine	3.8
<sup>99m</sup> Tc-DTPA	0.035
<sup>99m</sup> Tc-human serum albumin	0.018
<sup>99m</sup> Tc-lungaggregate	0.035
<sup>99m</sup> Tc-polyphosphate	0.036
<sup>99m</sup> Tc-sodium pertechnetate	0.037
<sup>99m</sup> Tc-stannous glucoheptonate	0.04
<sup>99m</sup> Tc-sulfur colloid	0.032
<sup>123</sup> I-sodium iodide (15% uptake)	0.032
<sup>131</sup> I-sodium iodide (15% uptake)	0.1
<sup>123</sup> I-rose bengal	0.13
<sup>131</sup> M-rose bengal	0.68

Courtesy of Dr. J. Kereikes, unpublished data, 1984

of cases from the 1950s through the 1980s have documented the induction of hypothyroidism and cretinism from doses of iodine-131 to the fetal thyroid.

Although pregnant women receive diagnostic x-rays occasionally, it is rare for them to be given radioactive isotopes. In general, physicians and patients alike are much more wary about nuclear medicine procedures than about diagnostic x-rays, even if dose levels may be similar. Never is this more true than in the case of pregnant or potentially pregnant women. Table 14.29 includes estimates of the dose to the embryo for selected radiopharmaceuticals.

### SUMMARY OF PERTINENT CONCLUSIONS

- Everyone is exposed to radiation from:
  - Unperturbed natural sources
  - Enhanced natural sources
  - Sources resulting from human **activity**, including medical x-rays.
- Natural background radiation comes from cosmic rays, terrestrial radiation from the earth's crust, and inhaled or ingested radioactivity.
- Cosmic-ray levels **vary** with altitude and latitude.
- Terrestrial radiation **levels Vary** widely with locality.
- Radon and its progeny result in irradiation of lung tissue with  $\alpha$ -particles; this is the largest source of natural radiation.
- Diagnostic radiology is the largest source of radiation resulting from human activities.

- About 200 million medical and about 100 million dental x-ray examinations are performed each year in the United States.
- About 10 million doses of radiopharmaceuticals are administered each year in the United States.
- The relative importance of natural background radiation compared with radiation from human activities can be assessed from the relevant annual average total effective doses, to the US population, which are:

Natural background, radon	2 mSv	200 mrems
Natural background, other	1 mSv	100 mrems
Medical diagnostic x-rays	0.39 mSv	30 mrems
Nuclear medicine	0.14 mSv	14 mrems
Consumer products	0.12 mSv	12 mrems
Rounded total	3.6 mSv	360 mrems

- Except for interventional radiology, the doses involved in diagnostic radiology do not result in *deterministic* effects; the risks are *stochastic* effects, i.e., carcinogenesis and hereditary effects.

### Computed Tomography

- The use of computed tomography has increased dramatically in the past decade.
- In 1997, there were about 20,000 CT scanners in the world, with an associated annual total of about 67 million CT procedures. The number of scanners per million population is much greater in the US than elsewhere.
- The multiple scan average dose ranges from 40 to 60 mGy (4 to 6 rad) for head scans to 10 to 40 mGy (1 to 4 rad) for body scans.
- CT scans involve relatively large effective doses since larger volumes of tissue are exposed to higher doses than occurs in common x-rays. The *effective dose* varies from a low of 0.34 mSv (34 mrems) when only part of the head is exposed, to a high of 7.8 mSv (780 mrems) for a routine multiple-slice chest CT.
- It is estimated that while CT comprises 4% of diagnostic procedures, it contributes 40% to the *collective effective dose*.
- Effective doses from CT scans are larger in small children than in adults.

### Effective Dose and Cancer

- The cancer risk to the individual is expressed in terms of the effective dose, the equivalent dose to the various organs and tissues exposed, multiplied by the appropriate tissue weighting factors (WT).
- The collective effective dose is the product of the effective dose and the number of individuals exposed. It gives an indication of the harm or "detriment" to an exposed population.
- The annual collective effective dose to the US population from diagnostic x-rays is about 92,000 person-Sv (9.2 million man-rems). This estimate is out of date, and therefore probably too low because of the increased use of CT, but may instead be too high because the population receiving x-rays is skewed with regard to age distribution as compared with the general population. Overall, it is the best we have.
- The weighted annual collective effective dose for nuclear medicine is 13,500 person-Sv (1.35 million man-rems). An even bigger allowance is necessary to correct for the older age distribution of nuclear medicine patients than of patients receiving diagnostic radiology.
- The "detriment" from diagnostic radiology and nuclear medicine may be calculated from the collective effective dose and the risk estimate of 4% per sievert for fatal cancer, 0.8% per sievert for nonfatal cancer, and 0.6% per sievert for serious heritable effects.

- Detriment includes an allowance for loss of quality of life, as well as for death.
- The practice of diagnostic radiology for 1 year in the United States may benefit about half of the population but may result in several thousand fatal cancers, several hundred non-fatal cancers, and several hundred heritable effects (mutations).
- The practice of nuclear medicine for 1 year in the United States may benefit 10 million patients but may result in several hundred fatal cancers, about 100 nonfatal cancers, and about 100 serious heritable effects.

### Interventional Procedures

- The 1990s have witnessed a major increase in high-dose fluoroscopically guided interventional procedures in medicine.
- Radiation doses to patients from interventional radiology and cardiology are in general much higher than from general diagnostic radiology.
- There is a risk of deterministic effects, such as early or late skin damage from interventional procedures. These include:
  1. Early transient erythema, which may occur in a matter of hours following doses of more than 2 Gy (200 rad). The main wave of erythema peaks at 10 days to 2 weeks and requires a larger dose of about 6 Gy (600 rad).
  2. Dry desquamation, which may occur after single doses of more than 10 Gy (1000 rad) due to depopulation of clonogenic cells in the epidermis.
  3. Moist desquamation, which requires doses exceeding 15 Gy (1500 rad) and is also due to depopulation of clonogenic cells in the epidermis. These effects may cause substantial discomfort, but provided they are not too severe, they will heal and clear up as the population of basal cells recovers.
  4. Temporary epilation, which may occur after doses of about 3 Gy (300 rad), with an onset at about 3 weeks and regrowth requiring 5 weeks or more. Epilation is permanent if the dose exceeds about 7 Gy (700 rad).
  5. Late effects in the skin, which include dermal atrophy, telangiectasia, and necrosis. These effects occur months to years after higher doses of radiation. Late effects may also develop after unusually severe early effects, in which case they are referred to as "consequential" late effects.
  6. Above a certain threshold, the risk of skin damage is a function of the AEP, the area exposure product, with units of Gy cm<sup>2</sup>.
  7. To put things into context, hundreds of thousands of patients each year in the US alone undergo interventional procedures, yet serious problems are rare; for example, about 40 injuries were reported to the FDA in 1994.
- Since most patients undergoing interventional procedures are older and suffer from life-threatening medical conditions, the possibility of stochastic effects (such as radiation-induced cancer) 10 to 20 years in the future is largely academic.

### Effects on the Embryo and Fetus

- For irradiation of the human in utero, the risk of severe mental retardation as a function of dose is apparently linear, with a risk coefficient as high as 40% per Sv (40% per 100 rems) at 8 to 15 weeks after conception and about four times lower at 16 to 25 weeks. The data are also consistent with a threshold of about 0.2 Sv (20 rems).
- Loss of IQ is estimated to be about 30 points per sievert (30 points per 100 rems).
- Every precaution should be taken to avoid exposure of a conceptus.
- Many x-ray exposures involve doses too small to pose a significant risk to the embryo or fetus.

- In the event of an accidental exposure of an unsuspected conceptus, the dose should be estimated carefully. Some believe that a dose exceeding 0.1 Sv (10 rems) during a sensitive period of gestation may be grounds for a therapeutic abortion. This dose is flexible and depends on many social factors, such as the religious beliefs of the parents or the number of children they already have.
- The effects of radionuclides on the developing embryo and fetus have been studied less than the effects of x-rays and are likely to be much more complex. Special care should be taken to avoid this problem.

#### Summary

- In the treatment of hyperthyroidism with iodine-131, the risk of death from radiation-induced leukemia or cancer is less than the risk of death from a general anesthetic used during surgery.
- The risks posed by diagnostic x-rays and nuclear medicine procedures are small compared with other hazards in an industrialized society, such as smoking cigarettes or driving an automobile.

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## Radiation Protection

THE ORIGINS OF RADIATION PROTECTION  
 ORGANIZATIONS  
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 RADIATION WEIGHTING FACTORS  
 EQUIVALENT DOSE  
 EFFECTIVE DOSE  
 COMMITTED EQUIVALENT DOSE  
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     COMMITMENT  
 SUMMARY OF QUANTITIES AND UNITS  
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 AS LOW AS REASONABLY ACHIEVABLE  
     (ALARA)

PROTECTION OF THE EMBRYO/FETUS  
 EMERGENCY OCCUPATIONAL EXPOSURE  
 EXPOSURE OF PERSONS YOUNGER THAN  
     18 YEARS OF AGE  
 EXPOSURE OF MEMBERS OF THE PUBLIC  
     (NONOCCUPATIONAL LIMITS)  
 EXPOSURE TO INDOOR RADON  
*DE MINIMIS* DOSE AND NEGLIGIBLE  
     INDIVIDUAL DOSE  
 RISKS ASSOCIATED WITH CURRENT  
     RECOMMENDED LIMITS  
 NATIONAL COUNCIL ON RADIATION  
     PROTECTION AND MEASUREMENTS  
     AND INTERNATIONAL COMMISSION  
     ON RADIATION PROTECTION  
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### THE ORIGINS OF RADIATION PROTECTION

At the Second International Congress of Radiology in Stockholm in 1928, member countries were invited to send representatives to prepare x-ray protection recommendations. The British recommendations were adopted because they were most complete: Guidelines on radiation protection had been set up in that country as early as 1915.

The 1928 congress set up the International Committee on X-Ray and Radium Protection, which after World War II was remodeled into two commissions that survive to this day:

The International Commission on Radiation Protection (ICRP)

The International Commission on Radiological Units and Measurements (ICRU)

The U.S. representative to this 1928 congress was Dr. Lauristor Taylor, who brought back to the United States the agreed radiation protection criteria and set up a national committee, the Advisory Committee on X-Ray and Radium Protection, under the auspices of the Bureau of Standards, which was perceived to be "neutral territory" by the various radiologic societies of the day. This committee operated until World War II; in 1946 it was renamed the National Council on Radiation Protection and Measurements (NCRP), eventually receiving a charter from Congress as an independent body to advise on matters pertaining to radiation protection in the United

States. NCRP reports to this day form the basis of radiation protection policy in the United States, though legal responsibility for the implementation of radiation safety is variously in the hands of the Nuclear Regulatory Commission, the Department of Energy, and state or city bureaus of radiation control.

## ORGANIZATIONS

The organization of radiation protection and the interrelation of the various committees whose reports are quoted deserve a brief explanation.

First, there are the committees that summarize and analyze data and suggest risk estimates for radiation-induced cancer and genetic effects. At the international level there is the United Nations Scientific Committee on the Effects of Atomic Radiation, usually known as UNSCEAR. This committee has wide international representation. Reports appeared in 1958, 1966, 1972, 1977, and 1982, with the latest report in 1988. The United States committee is appointed by the National Academy of Sciences and is known as the BEIR (Biological Effects of Ionizing Radiations) Committee. The first report appeared in 1956, when it was known as the BEAR (Biological Effects of Atomic Radiation) Committee. Subsequent reports appeared in 1972 (BEIR II), 1980 (BEIR III), and 1990 (BEIR V).

These committees are "scholarly" committees in the sense that if information is not available on a particular topic they do not feel compelled to make a recommendation. Because they do not serve an immediate pragmatic aim, they are not obliged to make a "best-guess" estimate if data are uncertain. For example, both committees declined to choose a value for the dose-rate effectiveness factor for carcinogenesis in the human (for which there are no data) and simply described a range of 2 to 10 based on animal studies.

Second, there are the committees that formulate the concepts for use in radiation protection and recommend maximum permissible levels. These committees serve more

pragmatic aims and therefore must make best estimates even if good data are unavailable. At the international level there is the ICRP, which (together with the International Commission on Radiological Units and Measurements) was established in 1928 after a decision by the Second International Congress of Radiology. In 1950, this commission was restructured and given its present name. The ICRP often takes the lead in formulating concepts in radiation protection and in recommending dose limits. As an international body it has no jurisdiction over anyone and can do no more than recommend; it has established considerable credibility, however, and its views carry great weight. Its most recent report is ICRP Publication No. 90, published in 1991.

In the United States there is the NCRP, chartered by Congress to be an "impartial" watchdog and consisting of 100 experts from the radiation sciences who are, therefore, not impartial at all. The NCRP often, but not always, follows the lead of ICRP. Their most recent report on dose limits (NCRP Report No. 116, published in 1992) differs from ICRP in several important respects. The ICRP and NCRP suggest dose limits and safe practices, but in fact neither body has any jurisdiction to enforce their recommendations.

In the United States, the Environmental Protection Agency (EPA) has responsibility for providing guidance to federal agencies; it is the EPA that sets, for example, the action level for radon. Each state can formulate its own regulations for x-rays and radiations produced by sources other than reactors. In agreement states, the Nuclear Regulatory Commission formulates rules for by-product materials from reactors; in other states, this responsibility falls on the U.S. Occupational Safety and Health Administration. The Department of Energy is responsible for radiation safety regulations at all of its facilities operated by contractors. Up to the present time, the various regulating bodies in the United States have accepted, endorsed, and used the reports issued by the NCRP, but they are not obligated to do so.

## QUANTITIES AND UNITS

The quantity used to measure the "amount" of ionizing radiation is the absorbed dose. This is defined to be the energy absorbed per unit mass, and its unit is joules per kilogram, which is given a special name, gray (Gy). The unit used in the past was the rad, defined to be an energy absorption of 100 ergs/g. Consequently, 1 Gy equals 100 rad.

## RADIATION WEIGHTING FACTORS

The probability of a stochastic effect, such as the induction of cancer or of heritable events, depends not only on the dose but also on the type and energy of the radiation; that is, some radiations are biologically more effective, for a given dose, than others. This is taken into account by weighting the absorbed dose by a factor related to the quality of the radiation. A radiation **weighting factor** is a dimensionless multiplier used to place biological effects (risks) from exposure to different types of radiation on a common scale. Radiation weighting factors are chosen by commissions such as the ICRP as representative of relative biological effectiveness, applicable to low doses and low dose rates, and for biological endpoints relevant to stochastic late effects. They can be traced ultimately to experimentally determined RBE values, but a large judgmental factor is in-

volved in their choice. The weighting factors recommended by the ICRP for different types of radiations, such as protons, neutrons, and alpha-particles, are listed in Table 15.1.

## EQUIVALENT DOSE

In radiologic protection, the **equivalent dose** is the product of the absorbed dose averaged over the tissue or organ and the radiation weighting factor selected for the type and energy of radiation involved. Thus,

$$\text{Equivalent dose} = \text{absorbed dose} \times \text{radiation factor}$$

If absorbed dose is measured in grays, the equivalent dose is in sieverts (Sv). If the absorbed dose is in rads, the equivalent dose is in rems. Although 1 Gy of neutrons does not produce the same biologic effect as 1 Gy of x-rays, 1 Sv of either neutrons or x-rays does result in equal biologic effects.

If a radiation field is made up of a mixture of radiations, the equivalent dose is the sum of the individual doses of the various types of radiations, each multiplied by the appropriate radiation weighting factor. Thus, if a tissue or organ were exposed to 0.15 Gy of cobalt-60 x-rays, plus 0.02 of 1-MeV neutrons, the equivalent dose would be

$$0.15 \times 1 + 0.02 \times 20 = 0.55 \text{ Sv}$$

TABLE 15.1. *Radiation Weighting Factors*

Type and Energy Range	Radiation Weighting Factor
Photons, all energies	1
Electrons and muons, all energies	1
Neutrons, energy <10 keV	5
10 keV to 100 keV	10
>100 keV to 2 MeV	20
>2 MeV to 20 MeV	10
>20 MeV	5
Protons, other than recoil protons, energy >2 MeV α-Particles, fission fragments, heavy nuclei	20

Data from International Commission on Radiation Units and Measurements: Recommendations. Report No. 60. New York, Pergamon Press, 1991.

## EFFECTIVE DOSE

If the body is uniformly irradiated, the probability of the occurrence of stochastic effects (cancer and heritable mutations) is assumed to be proportional to the equivalent dose, and the risk can be represented by a single value. In fact, truly uniform total-body exposures are rare, particularly if irradiation is from radionuclides deposited in tissues and organs. Sometimes, equivalent doses to various tissues differ substantially, and it is well established that different tissues vary in their sensitivities to radiation-induced stochastic effects. For example, it is difficult to produce heritable effects by irradiation of the head or hands! On the other

TABLE 15.2. *Tissue Weighting Factors*

Tissue or Organ	Tissue Weighting Factor
Gonads	0.20
Bone marrow (red)	0.12
Colon	0.12
Lung	0.12
Stomach	0.12
Bladder	0.05
Breast	0.05
Liver	0.05
Esophagus	0.05
Thyroid	0.05
Skin	0.01
Bone surface	0.01
Remainder	0.05

Data from International Commission on Radiation Units and Measurements: Recommendations. Report No. 60. New York, Pergamon Press, 1991.

hand, the thyroid and breast appear to be particularly susceptible to radiation-induced cancer. To deal with this situation, the ICRP introduced the concept of the **tissue weighting factor**, which represents the relative contribution of each tissue or organ to the total detriment resulting from uniform irradiation of the whole body. Table 15.2 lists the tissue weighting factors recommended by the ICRP.

The sum of all of the weighted equivalent doses in all the tissues or organs irradiated is called the **effective dose**.

The effective dose is

$$\text{£ absorbed dose} \times \text{WR} \times \text{WT}$$

for all tissues or organs exposed.

### COMMITTED EQUIVALENT DOSE

In the case of external irradiation the absorbed dose is delivered at the time of exposure; but for irradiation from internally deposited radionuclides, the total absorbed dose is distributed over time, as well as to different tissues in the body. The dose rate falls off, depending on the physical and biologic half-lives of the radionuclide.

To take into account the varying time distributions of dose delivery, the ICRP defined the **committed equivalent dose** as the integral over 50 years of the dose equivalent in a given tissue after intake of a radionuclide. This time

was chosen to correspond to the working life of a person. For radionuclides with effective half-lives up to about 3 months, the committed equivalent dose is essentially equal to the annual equivalent dose in the year of intake, but for radionuclides with longer effective half-lives it is greater, because it reflects the dose that will accrue over future years.

### COMMITTED EFFECTIVE DOSE

If the committed equivalent doses to individual organs or tissues resulting from the intake of a radionuclide are multiplied by the appropriate tissue weighting factors and then summed, the result is the **committed effective dose**.

### COLLECTIVE EQUIVALENT DOSE

The quantities referred to previously all relate to the exposure of a person. They become appropriate for application to the exposure of a group or population by the addition of the term *collective*. Thus the **collective equivalent dose** is the product of the average equivalent dose to a population and the number of persons exposed. There appears to be some confusion about the accepted name of the unit for collective dose equivalent in the new SI system of units. Some use *man-sievert*, presumably agreeing with the judgment of Sir Winston Churchill that "man embraces woman." The more liberated prefer the term *person-sievert*, which is used here. (The old unit was the man-rem.)

### COLLECTIVE EFFECTIVE DOSE

The **collective effective dose** is likewise the product of the average effective dose to a population and the number of persons exposed. The unit is again the *person-sievert* (formerly man-rem). An example is in order here. If 100 persons receive an average effective dose of 0.3 sievert (30 rems), the collective effective dose is 30 person-sieverts (3,000 man-rems).

These collective quantities can be thought of as representing the total consequences of exposure of a population or group. For example, the annual collective effective dose to

the U.S. population from diagnostic radiology is about 100,000 person-sieverts (10 million man-rems). Such collective quantities are much beloved by the bureaucrats because they make it possible to compare different activities or accidents, inasmuch each can be described by a single number. The danger is that the next step is to convert the collective dose into the number of cancers or heritable effects produced, which, of course, assumes proportionality between dose and biologic effect, which is seldom true. The quantities certainly are used widely to give a rough guide to the probability of cancer and hereditary effects in a population exposed to radiation, and in particular they can be used to compare approximately the impact of different types of radiation accidents in terms of the number of health effects that might arise in that population.

### COLLECTIVE EFFECTIVE DOSE COMMITMENT

In the case of a population ingesting radionuclides that deposit their dose over a prolonged period of time, the integral of the effective dose over the entire population out to

a period of 50 years is called the **collective effective dose commitment**.

### SUMMARY OF QUANTITIES AND UNITS

Table 15.3 is a summary of quantities and units described previously, showing how they build logically one on another. If on reading this section the reader gains the impression that the bureaucrats have taken over, it is because they have—at least in the field of radiation protection. An elaborate set of definitions has been produced based on the assumption of linearity between risk and dose. The whole business needs to be taken with a generous **pinch** of salt, because it is like a house of cards built as an inverted pyramid, based on somewhat shaky and flimsy premises that cannot be tested.

The concept of collective effective dose does allow a rough and quick estimate to be made of the potential health hazards to a population from, for example, an accidental release of radioactivity from a nuclear reactor. It must be emphasized again that these concepts can be used only under conditions in which it is *reasonable to assume linearity between risk*

TABLE 15.3. Quantities and Units Used in Radiation Protection

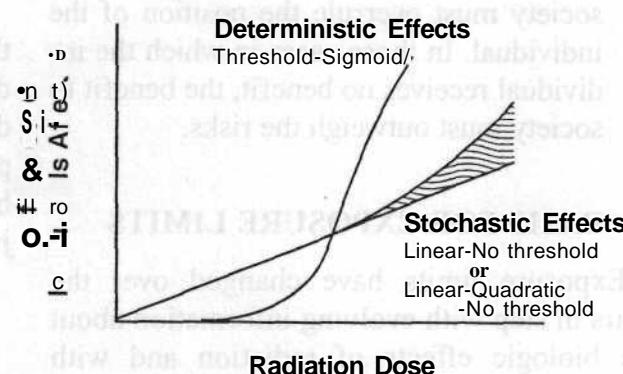
Quantity	Definition	New	Unit	Old
Absorbed dose For individuals	Energy per unit mass	gray	rad	
Equivalent dose	Average dose x radiation weighting factor	sievert	rem	
Effective dose	Sum of equivalent doses to organs and tissues exposed, each multiplied by the appropriate tissue, weighting factor	sievert	rem	
Committee equivalent dose	Equivalent dose integrated over 50 years (relevant to incorporated radionuclides)	sievert	rem	
Committed effective dose	Effective dose integrated over 50 years (relevant to incorporated radionuclides)	sievert	rem	
For populations				
Collective effective	Product of the average effective dose and the number of individuals exposed	person-sievert	man-rem	
Collective effective dose committed	Integration of the collective dose over 50 years (relevant to incorporated radionuclides)	person-sievert	man-rem	

and dose; that is, that risks are directly proportional to the summation of doses from different sources. Exposures that are within the administratively allowed dose limits may cause an increased incidence of stochastic effects, such as cancer and genetic mutations, but are much below the thresholds for early deterministic effects. In the case of larger accidental releases, in which doses to some persons might be high enough to exceed these thresholds to the point of causing early death, collective effective dose is an inappropriate quantity.

## OBJECTIVES OF RADIATION PROTECTION

As stated by the NCRP, the objectives of radiation protection are (1) to prevent clinically significant radiation-induced deterministic effects by adhering to dose limits that are below the apparent or practical threshold, and (2) to limit the risk of stochastic effects (cancer and heritable effects) to a reasonable level in relation to societal needs, values, and benefits gained. The difference in shape of the dose-response relationships for deterministic and stochastic effects is illustrated in Figure 15.1. The objectives of radiation protection can be achieved by reducing all exposure to as low as reasonably achievable (ALARA) and by applying dose limits for controlling occupational and general public exposures. For radiation-protection purposes, it is assumed that *the risk of stochastic effects is strictly proportional to dose without threshold, throughout the range of dose and dose rates of importance in radiation protection.* Furthermore, the probability of response (risk) is assumed to accumulate linearly with dose. This is not true at higher doses characteristic of accidents, in which more complex (nonlinear) dose-risk relationships may apply.

Given these assumptions, any selected dose limit has an associated level of risk. Consequently, it is necessary to justify any use of radiation in terms of a benefit to a person or to society.



**Figure 15.1.** The basic differences in the shape of the dose-response relationship for stochastic as opposed to deterministic effects. Deterministic effects (e.g., cataracts or mental retardation) show no threshold in dose; the severity of the effect increases with dose above this threshold, and the proportion of individuals rises rapidly with dose to 100%. The dose-response relationship is therefore sigmoid, after a threshold. Stochastic effects are all-or-nothing effects (e.g., cancer and heritable effects). The severity of the effect is not dose-related, though the probability of it occurring is. The increase with dose may be linear or linear-quadratic. There is no threshold, that is, no dose below which the probability of an effect is zero. The dose-response relationship is therefore linear, or linear-quadratic, with no threshold.

Justification of exposure is one of the basic principles of radiation protection. The concept was described in 1977 by the ICRP:

A practice involving exposure to radiation should produce sufficient benefit to the exposed individual or to society to offset the radiation detriment it causes.

This concept is sometimes difficult to put into practice in the variety of situations in which individuals are exposed:

1. In the case of patients, the diagnostic or therapeutic benefit should outweigh the risk of detriment.
2. In the case of occupational exposure, the radiation risk must be added to and compared with other risks in the workplace.
3. The most difficult situation is research, in which there are three groups of volunteers: those patients who may benefit, those patients who receive no benefit, and healthy volunteers. In these cases, the benefit to

society must overrule the position of the individual. In those cases in which the individual receives no benefit, the benefit to society must outweigh the risks.

### BASIS FOR EXPOSURE LIMITS

Exposure limits have changed over the years in step with evolving information about the biologic effects of radiation and with changes in the social philosophy within which recommended exposure limits are developed.

In the 1930s, the concept of a **tolerance dose** was used, a dose to which workers could be exposed continuously without any evident deleterious acute effects such as erythema of the skin.

By the early 1950s, the emphasis had shifted to late effects. The maximum permissible dose was designed to ensure that the probability of the occurrence of injuries was so low that the risk would be readily acceptable to the average person. At about that time, based on the results of genetic studies in *Drosophila* and mice, the occupational limit was reduced substantially and a limit for exposure of the public introduced. Subsequently, the genetic risks were found to be smaller and cancer risks larger than were thought at the time.

By the 1980s, the NCRP was comparing the probability of radiation-induced cancer death in radiation workers with annual accidental mortality rates in "safe" industries. Exposure standards therefore are necessarily based on a mixture of observed effects and judgments.

### LIMITS FOR OCCUPATIONAL EXPOSURE

The NCRP recommends the limits described in the following sections.

#### Stochastic Effects

1. The individual worker's lifetime effective dose should not exceed age in years  $\times$  10 mSv (1 rad), and no occupational exposure should be permitted until the age of 18 years.
2. The effective dose in any 1 year should not exceed 50 mSv (5 rems).

These limits apply to the sum of the effective dose from external radiation and the committed effective dose from internal exposures and are summarized in Table 15.4.

TABLE 15.4. Summary of Recommended Dose Limits

	NCRP	ICRP if Different
Stochastic effects: effective dose limits		
Cumulative		
Annual	10 mSv $\times$ age 50 mSv/y	20 mSv/y averaged over 5 years
Deterministic effects: dose equivalent limits		
for tissues and organs (annual)		
Lens of eye	150 mSv/y	
Skin, hands, and feet	500 mSv/y	
Embryo or fetus exposure: Effective dose limit	0.5 mSv/month	Total of 2 mSv to abdomen surface
Public exposure (annual)		
Effective dose limit, continuous or frequent exposure	1 mSv/y	
Effective dose limit, infrequent exposure	5 mSv/y	Averaged over 5 years
Dose equivalent limits of lens of eye, skin, and extremities	50 mSv/y	
Education and training exposure (annual)		
Effective dose limit	1 mSv/y	No statement
Dose equivalent limit for lens of eye, skin, and extremities	50 mSv/y	No statement
Negligible individual dose (annual)	0.01 mSv/y	No statement

Based on National Council on Radiation Protection and Measurements: Recommendations on Limits for Exposure to Ionizing Radiation. NCRP Report No. 116, Bethesda, MD, 1993; and International Commission on Radiation Protection: Recommendations. Report No. 60. New York, Pergamon Press, 1991.

### Deterministic Effects

1. 150 mSv (15 rems) for the lens of the eye
2. 500 mSv (50 rems) for localized areas of the skin and the hands and feet

These additional limits are required because the weighting factors for, for example, the hands and the feet are so small that huge doses could be given before cancer induction became a problem. Other deterministic effects are limiting at lower doses.

### AS LOW AS REASONABLY ACHIEVABLE

The dose limits referred to previously are all upper limits and subject to the concept of **ALARA** (*as low as reasonably achievable*). The recommendation that standard-setting committees would like to make for personnel protection is zero exposure. This is not feasible, however, if society is to realize the enormous benefits derived from the uses of radiations and radioactive materials.

Radiation is potentially harmful, and exposure to it should be monitored continually and controlled. No unnecessary exposure should be allowed. Equipment and facilities should be designed so that exposure of personnel and the public is kept to a minimum and not up to a standard. No exposure at all should be permitted without considering the benefits that may be derived from that exposure and the relative risks of alternative approaches.

Of course the ultimate problem is determining what is "reasonable." How much expense is justified to reduce the exposure of personnel by a given amount? As a rule of thumb in the nuclear-power industry in the United States, ALARA has a cash value of about \$1000 per 10 mSv (1 rem). If the exposure of one person to 10 mSv (1 rem) can be avoided by the expenditure of this amount of money, it is considered to be reasonable. If the cost is more, it is considered to be unreasonable, and the exposure is allowed. The figure of \$1000 per 10 mSv (1 rem) applies to low doses, if the risk involves an objective health detriment. At higher dose levels, at which the accumulation

of an additional exposure may threaten a worker's job if the lifetime dose limit is approached, the cash value of saving 10 mSv (1 rem) may be closer to \$10,000. This sort of choice seldom has to be made in a hospital setting, except in the purchase of remote after-loading equipment for brachytherapy.

### PROTECTION OF THE EMBRYO OR FETUS

The NCRP recommends a monthly limit of 0.5 mSv (0.05 rem) to the embryo or fetus once the pregnancy is declared. Once pregnancy has been declared, the ICRP recommends a limit of 2 mSv to the surface of the woman's abdomen (lower trunk) for the remainder of the pregnancy. These recommendations are essentially similar and are designed to limit the risk of mental retardation, other congenital malformations, and carcinogenesis. The NCRP and ICRP no longer recommend specific controls for occupationally exposed women *until* a pregnancy is declared.

Internally deposited radionuclides pose special problems for protection of the embryo or fetus. Some remain in the body for long periods of time, and the doses delivered to fetal organs are not well known for all radionuclides. Consequently, particular care should be taken to limit the intakes of radionuclides by pregnant women so that the equivalent dose to the embryo or fetus would not exceed the recommended limit.

### EMERGENCY OCCUPATIONAL EXPOSURE

Under normal conditions, only actions involving the saving of life justify acute exposures in excess of the annual effective dose limit. The use of volunteers for exposures during emergency actions is desirable. If possible, older workers with low lifetime accumulated effective doses should be chosen from among the volunteers. Exposure during emergency actions that do not involve the saving of life should be controlled, to the extent possible, at the occupational exposure limits. If this

cannot be accomplished, the NCRP and ICRP recommendation of 0.5 Sv (50 rems) should be applied.

If, for lifesaving or equivalent purposes, the exposure may approach or exceed 0.5 Sv (50 rems) to a large portion of the body, the worker not only needs to understand the potential for acute effects but also should have an appreciation of the substantial increase in his or her lifetime risk of cancer. If the possibility of internal exposures also exists, this should be taken into account.

### **EXPOSURE OF PERSONS YOUNGER THAN 18 YEARS OF AGE**

For educational and training purposes it may be necessary and desirable to accept occasional exposure of persons younger than the age of 18 years, in which case an annual effective dose limit of 1 mSv (0.1 rem) should be maintained.

### **EXPOSURE OF MEMBERS OF THE PUBLIC (NONOCCUPATIONAL LIMITS)**

The limitation of radiation exposure of members of the public from manmade sources is inevitably arbitrary, because it cannot be based on direct experience. The variety of risks of accident and death faced by members of the public every day vary greatly; the number ranges from  $10^{-4}$  to  $10^{-6}$  per year. Depending on their nature, these risks seem to be accepted without much thought. At the same time, everyone is exposed to natural background radiation of about 1 mSv (100 millirems) annually, excluding radon, which may result in a mortality risk of  $\frac{1}{Qr^A}$  to  $10^{-5}$  annually.

Based on these considerations, the recommended levels for manmade sources other than medical are as follows: For continuous (or repeated) exposure the annual effective dose equivalent should not exceed 1 mSv (0.1 rem). It is clear, however, that larger exposures to more limited groups of persons are not especially hazardous, provided they do not occur often to the same groups. Consequently, a max-

imum permissible annual effective dose equivalent of 5 mSv (0.5 rem) is recommended as a limit for infrequent exposure. Medical exposures are excluded from these limitations because it is assumed that they confer personal benefit to the exposed person and are extremely variable from one person to another.

Because some organs and tissues are not necessarily protected against deterministic effects in the calculation of effective dose, the hands and feet, localized areas of the skin, and the lens of the eye are also subject to an annual dose limit of 50 mSv (5 rem).

### **EXPOSURE TO INDOOR RADON**

Many homes in the United States and Europe contain an appreciable quantity of radon gas, which originates from the earth and enters the house through the basement. Insulating and sealing houses as a result of the escalating cost of heating oil in the 1970s exacerbated the radon problem, because a well-sealed house allows fewer exchanges of air with the outside and consequently results in a greater concentration of radon. Radon is a noble gas and is itself relatively nonhazardous because, if breathed in, it is breathed out again without being absorbed. In a confined space such as a basement, however, the decay of radon leads to the accumulation of progeny that are solids, which stick to particles of dust or moisture and tend to be deposited on the bronchial epithelium. These progeny emit  $\alpha$ -particles and cause intense local irradiation.

Radon levels vary enormously with different localities, depending on the composition of the soil and the presence of cracks, or fissures, in the ground, which allow radon to escape to the surface. There is the famous example of Stanley Watras, who wanted to work in a nuclear-power station but was turned away because the radiation monitors were set off as he entered the plant by the accumulation of progeny products deposited in his body and on his clothes that came from his home.

Indoor radon currently is perceived to be the most important problem involving radiation exposure of the public. In the United

States and most European countries, the mean radon concentration in homes is in the range 20 to 60 Bq/m<sup>3</sup> (0.5 to 1.6 pCi/L), with higher mean values of about 100 Bq/m<sup>3</sup> (2.7 pCi/L) in Finland, Norway, and Sweden. Converting radon concentrations into dose to the bronchial epithelium involves many uncertainties, because such conversion depends on the model used and the assumptions made. One widely used conversion factor equates an air concentration of 20 Bq/m<sup>3</sup> with an effective dose to the bronchial epithelium of 1 mSv/y (0.5 pCi/L corresponding to 100 millirems per year).

The EPA has set the "action level" at about 160 Bq/m<sup>3</sup> (4 pCi/L), suggesting that remedial action should be taken to reduce radon levels if they are higher than this. The action level is about four times the average radon concentration in homes. About 1 in 12 homes in the United States—about 6 million in all—have radon concentrations above this action level. This is the most stringent action level in the world. In Germany and Great Britain the action level is more than double this figure; in Finland, Sweden, and Canada it is five times higher.

The BEIR VI Committee of the National Academy of Sciences published a report on the health effects of radon in 1998. The committee's preferred central estimates, depending on which one of two models are used, are that about 1 in 10 or 1 in 7 of all lung-cancer deaths—amounting to 15,400 or 21,800 per year in the United States—can be attributed to radon. There are considerable uncertainties involved, and the number could be as low as 3,000 or as high as 32,000 each year. Most of the radon-related lung cancers occur among smokers, because of the synergism between smoking and radon. Among those who have never smoked, the committee's best estimate is that of the 11,000 lung cancer deaths each year, 1,200 or 2,900, depending on the model used, are radon-related. Of the deaths that can be attributed to radon, perhaps one third could be avoided by reducing radon in homes in which it is above the "action guideline level" of 148 Bq/m<sup>3</sup> (4 pCi/L) recommended by the EPA.

## DE MINIMIS DOSE AND NEGLIGIBLE INDIVIDUAL DOSE

Collective dose to a population has little meaning without the concept of *de minimis dose*. The idea is to define some very low threshold below which it would make no sense to make any additional effort to reduce exposure levels further. For example, suppose there is a release of radioactivity from a reactor that dissipates into the atmosphere, blows around the world, and eventually exposes many hundreds of millions of persons to very low doses. The doses may be so low that the biologic effects are negligible, but because the number of persons involved is so large, the product of the dose and the number of persons would dominate the collective dose. The term *de minimis* comes from the legal saying *De minimis non curat lex*, which roughly translates to, "The law does not concern itself with trifles."

Dr. Merril Eisenbud in an NCRP publication quotes this poem of dubious origin:

There was a young lawyer named Rex  
Who was very deficient in sex  
When charged with exposure  
He said with composure  
*De minimis non curat lex*

The concept of *de minimis* dose has been espoused by the NCRP in the form of negligible individual dose, defined here to be the dose below which further efforts to reduce radiation exposure to the person are unwarranted.

The NCRP considers an annual effective dose of 0.01 mSv (1 mrem) to be a **negligible individual dose**. This dose is associated with a risk of death between 10<sup>-6</sup> and 10<sup>-7</sup>, which is considered to be trivial compared with the risk of fatality associated with ordinary and normal societal activities and therefore can be dismissed from consideration of additional radioprotective measures.

## RISKS ASSOCIATED WITH CURRENT RECOMMENDED LIMITS

Risk estimates for radiation-induced cancer and hereditary effects are discussed in Chapters 10 and 11. They are summarized in Table

**TABLE 15.5.** Risk Estimates for Cancer and Hereditary Effects

Exposed Population	Fatal Cancer	Nonfatal Cancer	Severe Hereditary Effects	Total
Adult workers	4.0	0.8	0.8	5.6
Whole population	5.0	1.0	1.3	7.3

Data from International Commission on Radiation Units and Measurements: Recommendations. Report No. 60. New York, Pergamon Press, 1991.

15.5. The possible deleterious effects of long-term occupational exposure to radiation include the reduction of life expectancy (a combination of the probability of developing a fatal cancer and the number of years lost if it occurs) as well as the morbidity, that is, decreased quality of life, associated with nonfatal cancers and hereditary effects. The ICRP has coined the term *detriment* to cover all of these effects. The best estimate for the risk of fatal cancer in a working population exposed to a uniform total-body equivalent dose of 1 Sv at low dose rate is  $4 \times 10^{-2}$ . The contributions from nonfatal cancers and hereditary effects are more difficult to assess; the ICRP suggests 20% of the detriment from fatal cancers for each, that is,  $0.8 \times 10^{-2}$  per sievert.

The total detriment (life lost and quality of life impaired) amounts to about  $5.6 \times 10^{-2}$  per sievert. Recent surveys indicate that the average annual equivalent dose to monitored radiation workers with measurable exposures is about 2 mSv, which results in a total detriment per year

of less than  $2 \times 10^{-4}$ . This is comparable to the average death rate from fatal accidents in what are considered to be "safe" industries, such as trade and government service (Table 15.6). For those few persons who might receive doses close to the limit over an entire working life, the total detriment reaches 3.6% and is comparable to less safe industries such as construction or working in mines or quarries.

#### NATIONAL COUNCIL ON RADIATION PROTECTION AND MEASUREMENTS AND INTERNATIONAL COMMISSION ON RADIATION PROTECTION COMPARED

At the present time, there is a difference in the recommendations of the national and international bodies regarding the maximum permissible effective dose for occupational exposure (stochastic effects). The differences are highlighted in Table 15.4.

Both bodies recommend a maximum of 50 mSv (5 rems) in any one year, but the NCRP adds a lifetime cumulative limit of age x 10 mSv (1 rem), and the ICRP adds a limit of 20 mSv (2 rems) per year averaged over defined periods of 5 years.

The practical consequence of this difference is that a radiation worker starting at, for example, age 18 years can accumulate a larger dose under the NCRP recommendations in the early years up to 32 years of age but later in life could accumulate a larger dose under the ICRP recommendations. Under NCRP recommendations, a new radiation worker could receive 50 mSv (5 rems) in each of several consecutive years until the limit of age x 10 mSv (1 rem) kicks in. Under ICRP rules, the average cannot exceed 20 mSv (2 rems) per year

**TABLE 15.6.** Trends in Fatal Accident Rates (1976, 1989) for Workers in the United States

	Mean Rate $10^{-6} \text{y}^{-1}$	Mean Rate $10^{-6} \text{y}^{-1}$
All groups	142	90
Trade	64	40
Manufacture	89	60
Service	86	40
Government	111	90
Transport/public utilities	313	240
Construction	568	320
Mines and quarries	625	430
Agriculture (1973-1980)	541	400

Based on National Safety Council: Accident Facts 1976. Chicago, National Safety Council, 1977; and National Safety Council: Accident Facts 1989. Chicago, National Safety Council, 1990.

over a 5-year period, so one or two 50-mSv (5-rem) years would have to be followed by several years at very low exposure levels. If persons were exposed throughout their working lives to the maximum permissible dose, the excess risk of stochastic effects (cancer and hereditary effects) would be about the same under NCRP or ICRP recommendations. Under NCRP, a person occupationally exposed from 18 to 65 years of age could receive a total dose of 650 mSv (65 rems). Under the ICRP the same person could receive 940 mSv (94 rems), but less would be received between 18 and 32 years of age and more at later ages.

The NCRP scheme is less restrictive for a few workers in the nuclear-power industry who tend to receive large effective doses in their early years working on nuclear reactors,

Later in life, these persons tend to occupy supervisory or administrative positions and receive little if any dose. To cope with those who do not, NCRP has added the extra recommendation that this limit, age  $\times$  10 mSv (1 rem), can be relaxed *in individual cases after counseling*, if implementation of the recommendation would mean loss of a job.

It should be emphasized that few persons exposed occupationally in a medical setting receive doses anywhere near the limits recommended. Some interventional radiologists may well receive more than 50 mGy (5 rads) per year to a monitor worn outside the lead-rubber apron or to a monitor worn at neck level or on the forearm. But the recommended maximum permissible levels refer to **effective dose**, which takes into account the parts of the body exposed.

### SUMMARY OF PERTINENT CONCLUSIONS

- The objectives of radiation protection are to prevent clinically significant deterministic effects by keeping doses below the practical threshold and to limit the risk of stochastic effects (cancer and heritable effects) to a reasonable level in relation to societal needs, values, and benefits gained.
- Justification is one of the basic principles of radiation protection; a practice involving exposure to radiation should produce sufficient benefit to the exposed individual or to society to offset the radiation detriment it causes.
- Radiation weighting factors are approximate values of the relative biologic effectiveness, applicable to low doses and relevant to carcinogenesis and hereditary effects. Radiation weighting factors are chosen by the ICRP based on experimental relative biologic effectiveness values with a large judgmental factor.
- Equivalent dose is the product of absorbed dose and radiation weighting factor. The units are sieverts or rems for an absorbed dose in grays or rads.
- Tissue weighting factors reflect the susceptibility of different organs or tissues to carcinogenesis or hereditary effects.
- Effective dose is the sum of the weighted equivalent doses for all irradiated tissues and organs; the weighting factors represent the different risks of each tissue or organ for cancer or hereditary effects.
- Committed equivalent dose is the integral over 50 years of the equivalent dose after the intake of a radionuclide.
- Collective effective dose is a quantity for a population and is the sum of effective doses to all members of that population. The unit is person-sievert (man-rem).
- All radiation exposures are governed by the ALARA (as low as reasonably achievable) principle.
- The individual worker's lifetime effective dose should not exceed age in years  $\times$  10 mSv (1 rad)

- No occupational exposure should be permitted before 18 years of age.
- The effective dose in any 1 year should not exceed 50 mSv (5 rems).
- To limit deterministic effects, the dose limit to the lens of the eye is 150 mSv (15 rems), and the dose limit to localized areas of the skin, hands, and feet is 500 mSv (50 rems).
- Once a pregnancy is declared, the NCRP recommends a monthly limit of 0.5 mSv (0.05 rem) to the embryo or fetus.
- Specific controls for occupationally exposed women are no longer recommended until a pregnancy is declared.
- Internally deposited radionuclides pose a special problem for protection of the embryo or fetus; particular care should be taken to limit intake.
- Emergency occupational exposures normally justify doses in excess of the recommended limits only if life-saving actions are involved. Volunteers from among older workers with low lifetime accumulated effective doses should be chosen in emergencies in which the exposure may be up to 0.5 Sv (50 rems). If the exposure may exceed 0.5 Sv (50 rems), the worker should be counseled about the short-term and long-term possible consequences.
- For educational or training purposes it may sometimes be desirable to accept radiation exposures of persons younger than 18 years of age, in which case the annual effective dose limit of 1 mSv (0.1 rem) should be maintained.
- The annual effective dose limit/or *members of the public* is 1 mSv (0.1 rem), except for infrequent exposures, in which the limit may be 5 mSv (0.5 rem). Medical x-rays are excluded from these limitations because they are assumed to confer personal benefit.
- For deterministic effects, the dose limits for members of the general public are 50 mSv (5 rems) to the hands and feet, to localized areas of the skin, or to the lens of the eye.
- Indoor radon is perceived to be the most important problem involving radiation exposure of the general public. Remedial action in homes is recommended by the EPA if the radon concentration exceeds 160 Bq/m<sup>3</sup> (4 pCi/L). This represents a much higher cancer risk than the general public is allowed from other radiation sources and illustrates the equivocal views on radon.
- Negligible individual dose is the dose below which further expenditure to improve radiation protection is unwarranted. The negligible individual dose is an annual effective dose of 0.01 mSv (1 millirem), which carries a risk of between 10<sup>-6</sup> and 10<sup>-7</sup> of carcinogenesis or heritable effects.
- A uniform whole-body equivalent dose of 1 Sv to a working population is assumed to result in a total *detriment*, of about 5.6 x 10<sup>-2</sup> per sievert. This is made up of a risk of fatal cancer of 4 x 10<sup>-2</sup>, a risk of severe hereditary effects of 0.8 x 10<sup>-2</sup> per sievert, and a risk of nonfatal cancer of 0.8 x 10<sup>-2</sup> per sievert.
- The average annual equivalent dose to monitored radiation workers is about 2 mSv. This involves a total detriment of about 2 x 10<sup>-4</sup>, which is comparable to the annual risk of a fatal accident in a "safe" industry, such as trade or government service.
- The NCRP and ICRP differ in two important recommendations:
  1. *The effective dose limit for occupational exposure (stochastic effects)*: The NCRP recommends a lifetime cumulative limit of age x 10 mSv (1 rem) with a limit in any year of 50 mSv (5 rems). The ICRP recommends a limit of 20 mSv (2 rems) per year averaged over defined periods of 5 years, with a limit in any year of 50 mSv (5 rems).
  2. *The dose limit to the developing embryo or fetus once a pregnancy is declared*. The NCRP recommends a monthly limit of 0.5 mSv (0.05 rem) to the embryo or fetus. The ICRP recommends a limit of 2 mSv (0.2 rem) to the surface of the woman's abdomen for the remainder of pregnancy.

## GLOSSARY OF TERMS

**absorbed dose** The energy imparted to matter by ionizing radiation per unit mass of irradiated material at the place of interest. The unit is the gray (Gy), defined to be an energy absorption of 1 J/kg. The old unit was the rad, defined to be an energy absorption of 100 ergs per gram.

**ALARA** As low as reasonably achievable, economic and social factors being taken into account. This is identical to the principle of optimization of protection used by the ICRP.

**annual limit on intake** The activity of a radionuclide taken into the body during a year that would provide a committed equivalent dose to a person, represented by a reference "man," equal to the occupational dose limit set by recommending and regulating bodies. The annual limit normally is expressed in becquerels (Bq).

**becquerel (Bq)** The special name for the unit of activity.  $1 \text{ Gbq} = 3.7 \times 10^{10} \text{ Bq} = 1 \text{ Ci}$ .

**collective effective dose** Applies to a group of persons and is the sum of the products of the effective dose and the number of persons receiving that effective dose.

**collective equivalent dose** Applies to a group of persons and is the sum of the products of the equivalent dose and the number of persons receiving that equivalent dose.

**committed effective dose** The sum of the committed organ or tissue equivalent doses resulting from an intake multiplied by the appropriate tissue weighting factors.

**committed equivalent dose** The equivalent dose averaged throughout a specified tissue in the 50 years after intake of a radionuclide into the body.

**deterministic effects** Effects for which the severity of the effect in affected persons varies with the dose and for which a threshold usually exists. These were formerly known as *nonstochastic effects*. An example is a cataract.

**effective dose** The sum over specified tissues of the products of the equivalent dose in a tissue and the appropriate tissue weighting factor for that tissue.

**equivalent dose** A quantity used for radiation-protection purposes that takes into account the different probability of effects that occur with the same absorbed dose delivered by radiations of different quality. It is defined as the product of the averaged absorbed dose in a specified organ or tissue and the radiation weighting factor. The unit of equivalent dose is the sievert (Sv).

**genetically significant dose** The dose to the gonads weighted for the age and sex distribution in those members of the population expected to have offspring. The genetically significant dose is measured in sieverts (rems).

**gray (Gy)** The special name for the SI unit of absorbed dose, kerma, and specific energy imparted.  $1 \text{ Gy} = 1 \text{ J/kg}$ . One gray also equals 100 rad.

**negligible individual dose** A level of effective dose that can be dismissed as insignificant and below which further efforts to improve radiation protection are not justified. The recommended negligible individual dose is 0.01 mSv/y.

**nonstochastic effects** Previous term for *deterministic effects*.

**nuclide** A species of atom having a specified number of neutrons and protons in its nucleus.

**optimization** This has the same meaning as ALARA.

**organ or tissue weighting factor** A factor that indicates the ratio of the risk of stochastic effects attributable to irradiation of a given organ or tissue to the total risk if the whole body is uniformly irradiated. Organs that have a large tissue weighting factor are those that are susceptible to radiation-induced carcinogenesis (such as the breast or thyroid) or to heritable effects (the gonads).

**rad** The old unit for absorbed dose, kerma, and specific energy imparted. One rad is 0.01 J absorbed per kilogram of any material (also defined as 100 ergs per gram). The term is being replaced by gray:  $1 \text{ rad} = 0.01 \text{ Gy}$ .

**radiation weighting factor** A factor used for radiation-protection purposes that ac-

counts for differences in biologic effectiveness between different radiations. The radiation weighting factor is independent of the tissue weighting factor.

**relative biologic effectiveness** A ratio of the absorbed dose of a reference radiation to the absorbed dose of a test radiation to produce the same level of biological effect, other conditions being equal. It is the quantity that is measured experimentally.

**rem** The old unit of equivalent dose or effective dose. It is the product of the absorbed dose in rad and modifying factors and is being replaced by the sievert.

**sievert (Sv)** The unit of equivalent dose or effective dose in the SI system. It is the product of absorbed dose in gray and modifying factors; 1 Sv = 100 rems.

**stochastic effects** Effects for which the probability of their occurring, rather than their severity, is a function of radiation dose without threshold. More generally, stochastic means random in nature.

**working level** The amount of potential oc-particle energy in a cubic meter of air that results in the emission of  $2.08 \times 10^{-5}$  J of energy.

**working level month** A cumulative exposure, equivalent to exposure to one working level for a working month (170 hours), that is,  $2 \times 10^{-5} \text{ J-nr}^3 \times 170 \text{ h} = 3.5 \times 10^{-3} \text{ J-h-nr}^3$ .

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# 16

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## Molecular Techniques in Radiobiology

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### RNA AND DNA

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### HISTORICAL PERSPECTIVES

Recombinant DNA technology has revolutionized research in biology. It allows questions to be asked that, a few years ago, were unthinkable. It is also a technology that is moving fast; we are in the midst of a whirlwind revolution, so that anything written in a book is likely to be out of date before it appears in print. This technology is invading every field of biologic research, and radiobiology is no exception. To keep abreast with developments in the field it is essential to know what recombinant DNA technology is and how it works. A detailed description is beyond the scope of this book; for a more exhaustive account, the interested reader is referred to several excellent volumes that have appeared in recent years and are listed in the bibliography. The goal here is to provide an overview and to illustrate the way in which recombinant techniques have been used to solve specific problems in radiobiologic research.

The birth of molecular biology could be ascribed to the one-page publication in *Nature* in 1953 by Watson and Crick, describing the structure of DNA. In short order, this work led the way to breaking the genetic code and understanding the process of transcription of DNA to messenger RNA (mRNA) and the translation of mRNA into proteins. At about the same time, in the late 1940s and early 1950s, Linus Pauling realized that three-dimensional structures were formed as amino acids were folded into proteins, and that function was related to structure. The whole concept emerged that the sequence of bases, which coded for a protein, ultimately determined function.

These remarkable discoveries were followed by a period of limited progress focusing mainly on simple systems such as viruses, bacteriophages, and bacteria, until new tools and techniques to work with DNA were perfected.

The start of recombinant DNA technology was the first successful cloning experiment of

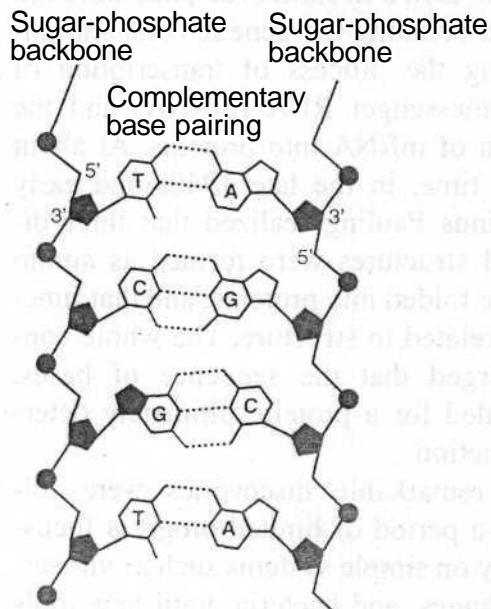
Stanley Cohen, in which he joined two DNA fragments together (a plasmid containing a tetracycline resistance gene with a kanamycin resistance gene), introduced this recombinant molecule into *Escherichia coli*, and demonstrated that the bacteria now had dual antibiotic resistance.

This simple experiment was only possible because of the simultaneous development of several techniques for cutting DNA with restriction enzymes and joining the fragments with ligases, and using *E. coli* as a host with the ability to take up foreign DNA through the use of plasmid vectors. This was followed quickly by the development of methods to sort pieces of DNA and RNA by size, using gel electrophoresis and blotting. The stage was set for an explosion of knowledge.

What follows is a brief and simplified description of these techniques and those that followed, together with some examples of their application to problems in radiobiology.

### THE STRUCTURE OF DNA

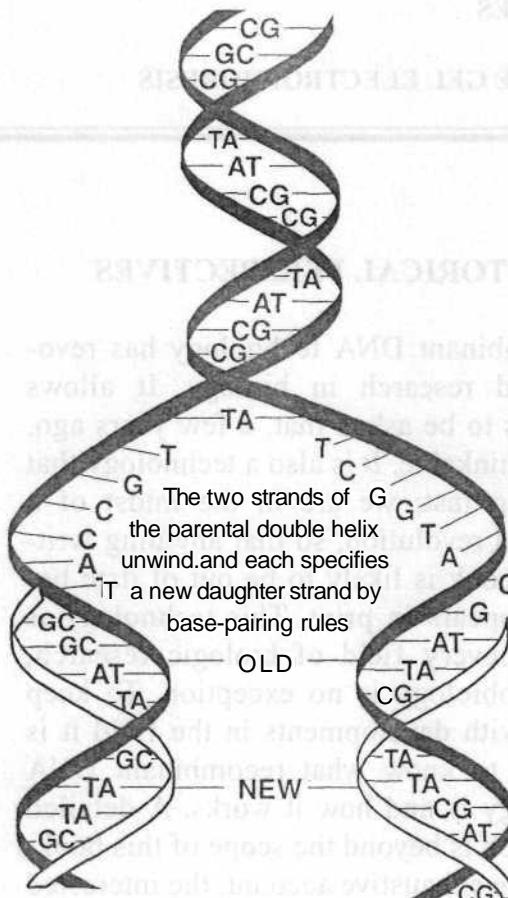
The structure of DNA arrived at by Crick and Watson is elegant in its simplicity. The mole-



**Figure 16.1.** The DNA double helix is held together by hydrogen bonds between base pairs. These are shown as dotted lines in the figure.

cule is composed of two antiparallel helices, looking rather like a gently twisted ladder. The rails of the ladder, which run in opposite directions, contain units of deoxyribose sugar alternating with a phosphate. Each rung is composed of a pair of nucleotides, a base pair held together by hydrogen bonds (Fig. 16.1). There is a complementary relationship between the bases: adenine always pairs with thymine, cytosine always pairs with guanine. Thus the nucleotide sequence of one strand of the DNA helix determines the sequence of the other.

This structure simply explains the way in which a DNA molecule replicates during cell division, so that each progeny cell receives an identical set of instructions. The hydrogen



**Figure 16.2.** The complementary nature of DNA is at the heart of its capacity for self-replication. The two strands of the parental DNA unwind and the hydrogen bonds break. Each strand then becomes a template to specify a new daughter strand obeying the base-pairing rules.

bonds between the base pairs break, allowing the DNA ladder to unzip (Fig. 16.2). Each half then constitutes a template for the reconstruction of the other half. Two identical DNA molecules result, one for each progeny cell.

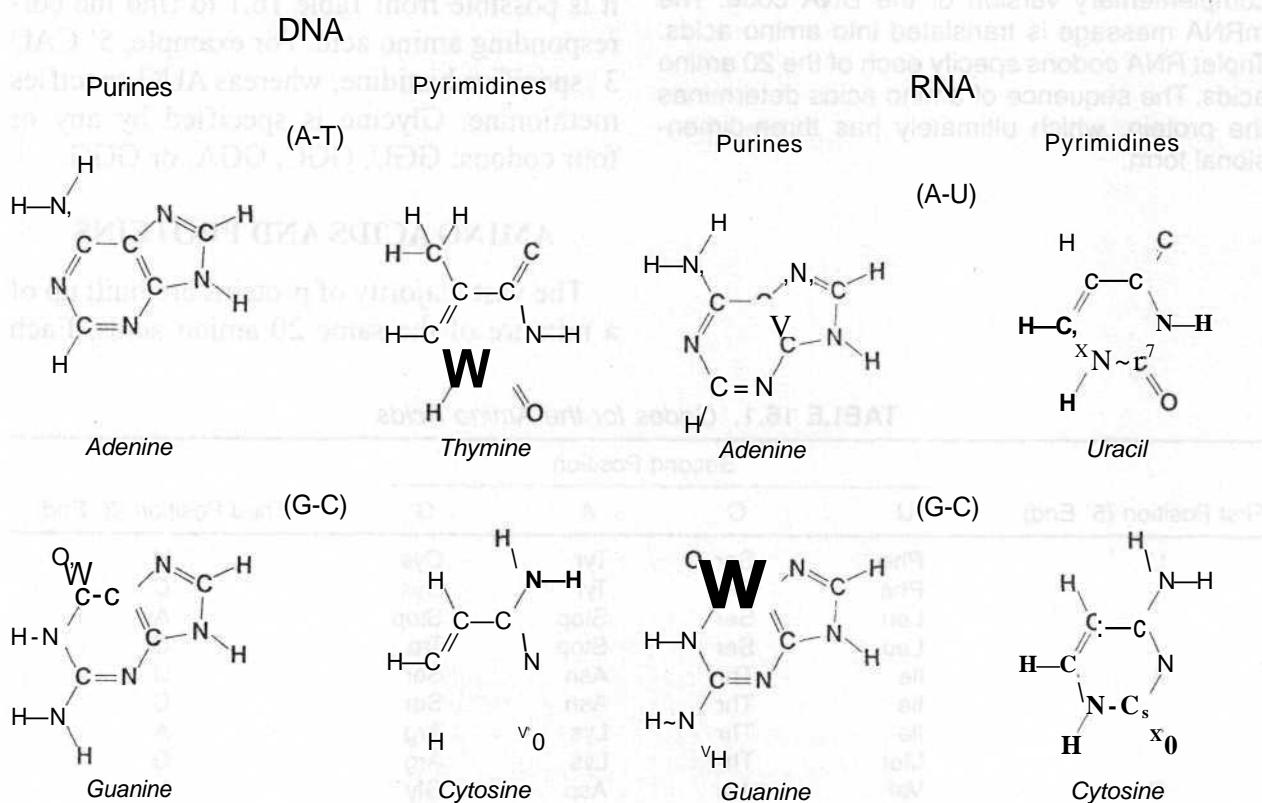
### RNA AND DNA

Unlike DNA, which is located primarily in the nucleus, RNA is found in abundance in the cytoplasm as well as the nucleus. Within the nucleus, RNA is concentrated in the *nucleoli*, dense granules attached to chromosomes. The sugar molecule in RNA is a *ribose* (hence its name, *ribonucleic acid*), whereas in DNA the sugar molecule is a *deoxyribose* (hence its name, *deoxyribonucleic acid*). In both DNA and RNA, the

bases are made up of two *purines* and two *pyrimidines*. The two purines, *adenine* and *guanine*, as well as the pyrimidine *cytosine* are common to both DNA and RNA. However, although *thymine* is found only in DNA, the structurally similar pyrimidine *uracil* appears in RNA (Fig. 16.3).

### TRANSCRIPTION AND TRANSLATION

The flow of genetic information from DNA to protein requires a series of steps. In the first step, the DNA code is *transcribed* in the nucleus into mRNA. Only the exons of the DNA are transcribed (Fig. 16.4). The mRNA transcript associates with a ribosome, at which, with the help of ribosomal RNA and transfer RNA (tRNA), the mRNA message is translated into a protein.



**Figure 16.3.** Illustrating the pairing of complementary bases in DNA and RNA. **Left:** DNA contains the purines adenine (A) and guanine (G), as well as the pyrimidines thymine (T) and cytosine (C). A purine always pairs with a pyrimidine, specifically, A pairs with T and G pairs with C. **Right:** RNA contains uracil (U) instead of thymine. In this case A pairs with U and G pairs with C.

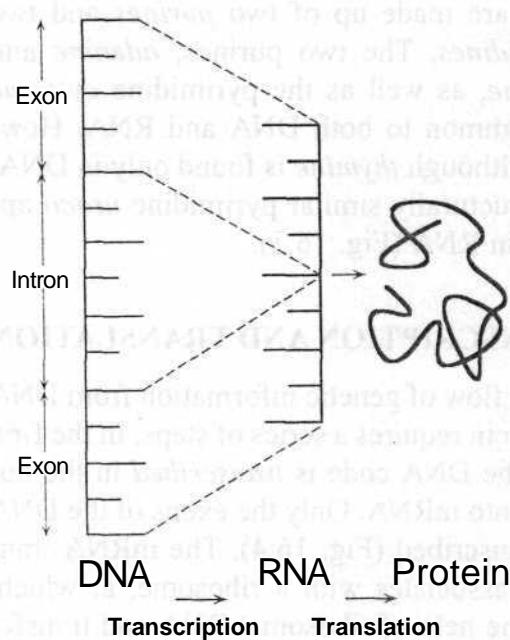


Figure 16.4. Illustration of the process of transcription and translation. The "information" in DNA is linear, consisting of combinations of the four nucleotides adenine, guanine, cytosine, and thymine. The information is transcribed into mRNA (messenger RNA), which in turn is a complementary version of the DNA code. The mRNA message is translated into amino acids. Triplet RNA codons specify each of the 20 amino acids. The sequence of amino acids determines the protein, which ultimately has three-dimensional form.

## THE GENETIC CODE

The code was cracked by 1966. Triplet mRNA sequences specify each of the amino acids. Because there are four bases, the number of possibilities for a three-letter code is  $4 \times 4 \times 4$ , or 64. There are only 20 amino acids, however; consequently, more than one triplet can code for the same amino acid—that is, there is redundancy in the code. Because nearly all proteins begin with the amino acid methionine, its codon (AUG) represents the "start" signal for protein synthesis. Three codons for which there are no naturally occurring tRNAs—UAA, UAG, and UGA—are "stop" signals that terminate translation. Only methionine and tryptophan are specified by a unique codon; all other amino acids are specified by two or more different codons. As a consequence of this redundancy, a single-base change in RNA does not necessarily change the amino acid coded for.

Given the position of the bases in a codon, it is possible from Table 16.1 to find the corresponding amino acid. For example, 5' CAU 3' specifies histidine, whereas AUG specifies methionine. Glycine is specified by any of four codons: GGU, GGC, GGA, or GGG.

## AMINO ACIDS AND PROTEINS

The vast majority of proteins are built up of a mixture of the same 20 amino acids. Each

TABLE 16.1. Codes for the Amino Acids

First Position (5' End)	Second Position				Third Position (3' End)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
A	Ile	Thr	Asn	Ser	U
	He	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G

polypeptide chain is characterized by a unique sequence of its amino acids. Chain lengths vary from 5 to more than 4,000 amino acids. Most proteins contain only one polypeptide chain, but there are others that are formed through the aggregation of separately synthesized chains that have different sequences. Though the vast majority of proteins are enzymes (*i.e.*, they act as catalysts, inducing chemical changes in other substances but themselves remaining apparently unchanged), many have structural roles as well. The essential fabric of both nuclear and plasma membranes is formed of proteins.

Once a polypeptide chain is synthesized from a string of amino acids, it tends to fold up into a three-dimensional form, the shape of which is governed by the weak chemical interactions between the side groups of the amino acids. Each three-dimensional shape is unique to the amino acid sequence. The shape of a protein is the key to its function.

## RESTRICTION ENDONUCLEASES

Restriction enzymes are endonucleases found in bacteria that have the property of recognizing a specific DNA sequence and cleaving at or near that site. These enzymes can be grouped into three categories, types I, II, and III. The restriction enzymes commonly used are of type II, meaning that they have endonuclease activity only (*i.e.*, they cut the DNA, without modification) at a predictable site within or adjacent to the recognition sequence. Types I and III have properties that make them impractical for use in molecular biology.

More than a thousand type II enzymes have been isolated, and more than 70 are commercially available. Some examples are shown in Table 16.2. They are named according to the following system:

1. The first letter comes from the genus of the organism from which the enzyme was isolated.
2. The second and third letters follow the organism's species name.
3. If there is a fourth letter, it refers to a particular strain of the organism.

TABLE 16.2. Examples of Type II Restriction Enzymes

Enzyme	Element of Terminology	Meaning
<i>HindW</i>	<i>H</i>	Genus <i>Haemophilus</i>
	<i>in</i>	Species <i>influenzae</i>
	<i>d</i>	Strain Rd
	<i>III</i>	Third endonuclease isolated
<i>EcoRI</i>	<i>E</i>	Genus <i>Escherichia</i>
	<i>co</i>	Species <i>coli</i>
	<i>R</i>	Strain RY13
	<i>I</i>	First endonuclease isolated
<i>BamUI</i>	<i>S</i>	Genus <i>Bacillus</i>
	<i>am</i>	Species <i>amyloliquefaciens</i>
	<i>H</i>	Strain H
	<i>I</i>	First endonuclease isolated

4. The roman numerals, as often as not, refer to the order in which enzymes were discovered, although the original intent was that it would indicate the order **in** which enzymes of the same organism and strain are eluted from a chromatography column.

Restriction endonucleases scan the DNA molecule, stopping if they recognize a particular nucleotide sequence. The recognition sites are short, four to eight nucleotides, and usually read the same **in** both directions, forward and backward, which is termed a *palindromic sequence*. Some endonucleases, such as *Hindll*, for example, produce blunt-ended fragments because they cut cleanly through the DNA, cleaving both complementary strands at the same nucleotide position, most often near the middle of the recognition sequence. Other endonucleases cleave the two strands of DNA at positions two to four nucleotides apart, creating exposed ends of single-stranded sequences. The commonly used enzymes *EcoRI*, *BamHL*, and *Hindlll*, for example, leave 5' overhangs of four nucleotides, which represent "sticky" ends, very useful for making recombinant molecules. Table 16.3 shows the recognition sequence and point of cutting of five commonly used restriction enzymes. This specificity is the same, regardless of whether the DNA is from a bacterium, a plant, or a human cell.

TABLE 16.3. Specificities of Few Typical Restriction Endonucleases

Enzyme End	Organism	Recognition Sequence <sup>a</sup>	Blunt or Sticky
EcoRI	<i>Escherichia coli</i>	GAATTC	Sticky
BamHI	<i>Bacillus amyloliquefaciens</i>	GGATCC	Sticky
BglIIW	<i>Bacillus globigii</i>	AGATCT	Sticky..
PvuI	<i>Proteus vulgaris</i>	CGATCG	Sticky
PvuW	<i>Proteus vulgaris</i>	CAGCTG	Blunt
HindIII	<i>Haemophilus influenzae R<sub>1</sub></i>	AAGCTT	Sticky
HinII	<i>Haemophilus influenzae R<sub>1</sub></i>	GANTC	Sticky
Sau3A	<i>Staphylococcus aureus</i>	GATC	Sticky
AluI	<i>Arthrobacter luteus</i>	AGCT	Blunt
TaqI	<i>Thermus aquaticus</i>	TCGA	Sticky
HaeIII	<i>Haemophilus aegyptius</i>	GGCC	Blunt
NotI	<i>Nocardia otitidis-caviae</i>	GCGGCCGG	Sticky

<sup>a</sup>The sequence shown is that of one strand given in the 5' to 3' direction. Only one strand is represented.

Most restriction recognition sites have symmetry in that the sequence on one strand is the same as on the other. For example, *EcoRI* recognizes the sequence 5' GAATTC 3'; the complementary strand is also 5' GAATTC 3'. *EcoRI* cuts the DNA between the G and A on each strand, leaving a 5' single-strand sequence of AATT on each strand. The strands are complementary. Therefore, all DNA fragments generated with *EcoRI* are complementary and can "base pair" with each other. This is illustrated in Figure 16.5.

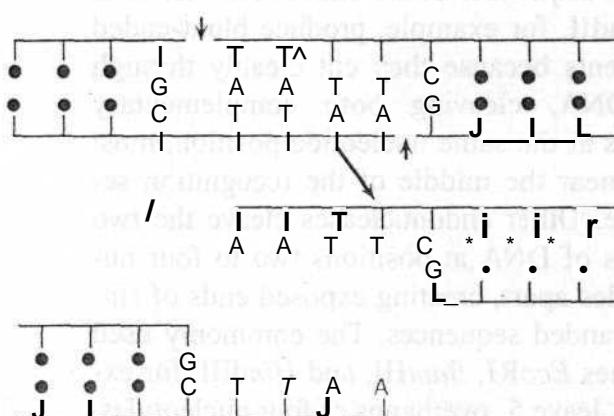


Figure 16.5. Illustration of how some endonucleases cleave each strand of the DNA off-center in the recognition site, creating fragments with exposed ends of short, single-stranded sequences. These "sticky" ends are extremely useful in making recombinant molecules, because they rejoin only with complementary sequences.

## VECTORS

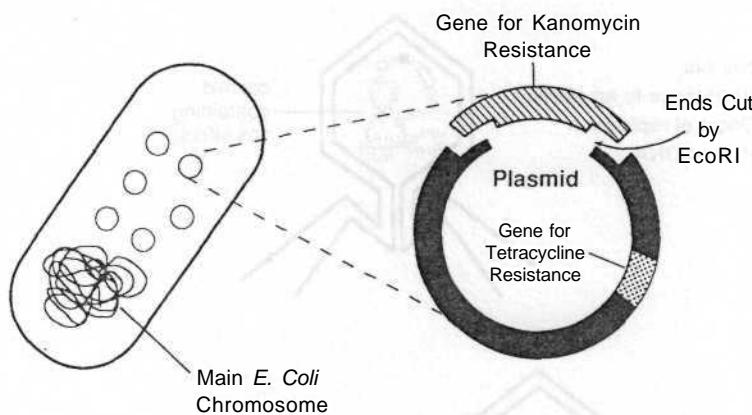
A vector is a **self-replicating** DNA molecule that has the ability to carry another foreign DNA molecule into a host cell. In the context of this chapter, the object of the exercise is usually to insert a fragment of human DNA (perhaps containing a gene of interest) into a bacterium so that it can be replicated and grown into quantities suitable for study.

There are many types of vectors:

1. Plasmids
2. Bacteriophage X
3. Cosmids
4. Yeast artificial chromosomes
5. Viruses

### Plasmids

The simplest bacterial vectors are **plasmids**, which are circular DNA molecules that can exist and replicate inside a bacterium, independent of the host chromosome. A piece of foreign DNA can be inserted into a plasmid, which in turn is introduced into a bacterium. As the bacterium grows and replicates, so, too, does the foreign DNA. The plasmid also contains a gene for resistance to an antibiotic (e.g., ampicillin), so that if the bacteria are subsequently grown in a culture medium containing antibiotic, only those bacteria that have taken up a plasmid **survive** and replicate. This is illustrated in Figure 16.6.



**Figure 16.6.** A plasmid is the simplest bacterial vector, that is, a means of carrying foreign DNA sequences into *Escherichia coli*. A plasmid is a circular DNA molecule, capable of autonomous replication, that typically carries one or more genes encoding an antibiotic resistance. Foreign DNA (e.g., from a human cell) also can be incorporated into the plasmid. If inserted into a bacterium, the plasmid replicates along with the main chromosome.

It is a relatively simple matter, subsequently, to harvest the recombinant plasmids. There are two limitations to this technique. First, plasmids are useful only for relatively small DNA inserts up to about 10,000 base pairs (bp). Second, the plasmids do not transfect into bacteria with high efficiency.

### Bacteriophage A,

Bacteriophages are bacterial viruses. The bacteriophage most commonly used as a cloning vector is **bacteriophage X**. It has two advantages compared with other vectors. As a bacteriophage particle, bacteriophage X can infect its host at a much higher efficiency than a plasmid, and it can accommodate a large range of DNA fragments, from a few to up to 24,000 bp, depending on the specific vector used. Many vectors derived from bacteriophage X exist. Some have been modified to be used to clone small DNAs, usually cDNAs, and some have been modified to clone large DNA molecules. If bacteriophage A, is used to clone large DNA molecules, the central portion of the bacteriophage DNA is deleted. This is to allow the foreign DNA to be accommodated within the bacteriophage particle, which has an upper limit of 55,000 bp. Once the bacteriophage DNA is ligated with the DNA to be cloned, the total DNA is mixed with extracts containing empty bacteriophage

particles. The ligated DNA is taken up into the bacteriophage, which is then used to infect *E. coli*.

To insert itself into the *E. coli* chromosome, it circularizes by the base pairing of the complementary single-strand tails that exist at its two ends—the **cos** sites. The resulting circular X DNA then recombines into the *E. coli* chromosome.

If part of the wild-type DNA of the bacteriophage is removed, room can be made for a piece of human DNA to be inserted, again with a gene that confers resistance to an antibiotic to allow selection. The bacteriophage then can be used to infect bacteria that multiply their own DNA as well as the integrated piece of human DNA. The bacteriophage X accommodates DNA inserts up to about 24,000 bp.

### Cosmids

A **cosmid** is a plasmid that contains a cos site. This is the sequence within bacteriophage that leads to its encapsulation within a bacteriophage particle. Cosmid vectors can accommodate up to 45 kilobases (kb) of DNA, which is packaged within a bacteriophage particle for efficient transfer into a bacterial cell. Once in the cell, cosmids grow like plasmids. Cosmids contain an antibiotic resistance gene to allow selection of infected cells. The use of cosmids is illustrated in Figure 16.7.

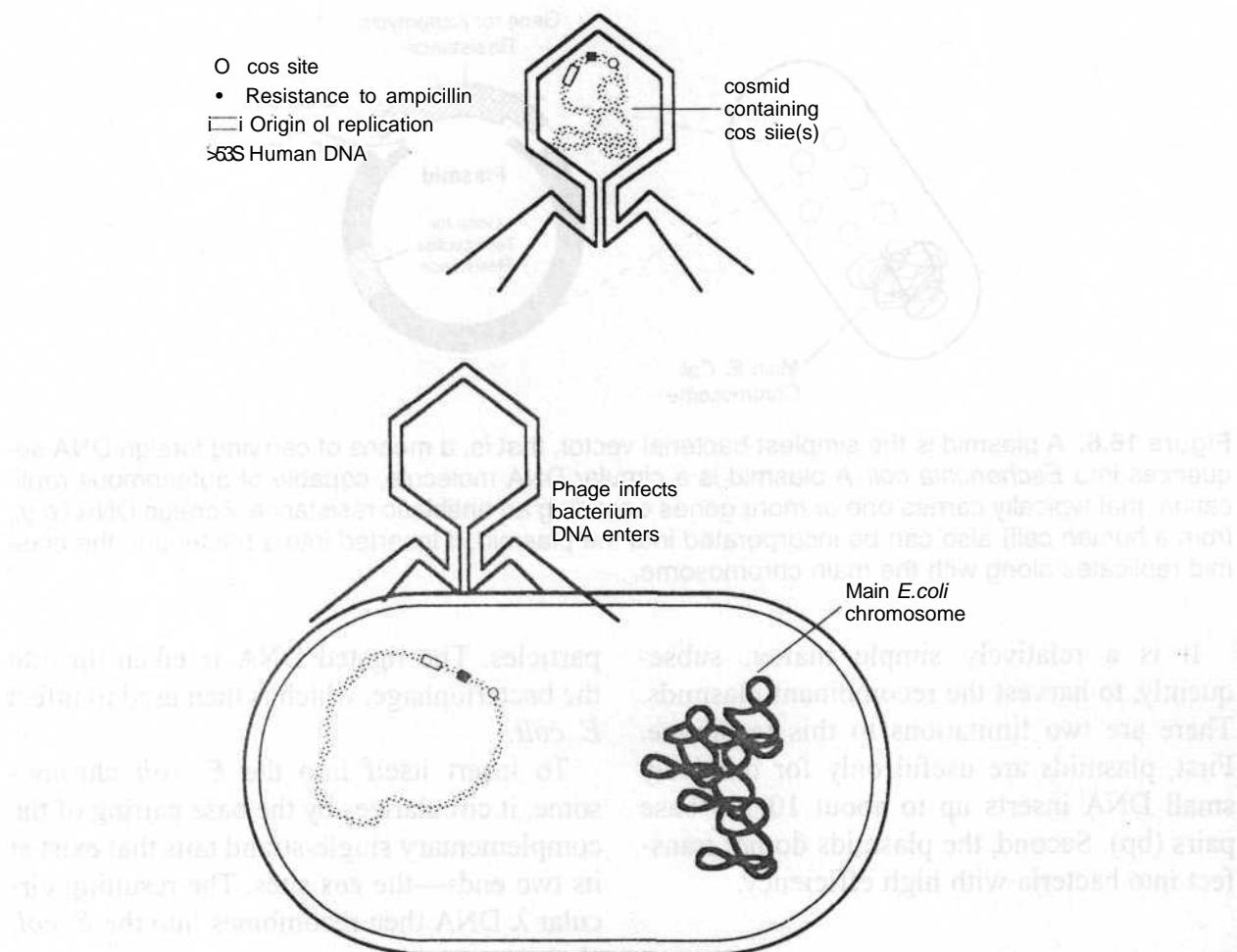


Figure 16.7. A bacteriophage is a virus that infects bacteria. It represents a much more efficient means of inserting foreign DNA into a bacterium than using a plasmid. If part of the wild-type DNA is removed, room can be made for a piece of "foreign" DNA, for example, from a human cell, as well as a gene that confers resistance to an antibiotic to allow selection. The DNA of the bacteriophage replicates along with that of the bacterium.

### Yeast Artificial Chromosomes

**Yeast artificial chromosomes** represent a recent development. Basically, a yeast artificial chromosome is a vector containing a centromere and telomeres, all of which are incorporated into large DNA fragments (up to 106 bp). These DNAs are introduced into the yeast *Saccharomyces cerevisiae*, in which they replicate as a chromosome.

### Viruses

**Viruses** are highly efficient vectors for introducing foreign genes into mammalian cells. SV40 was the first one employed, but it is limited in its usefulness. **Retroviruses** are ideal vectors for introducing genes into mammalian cells in a stable fashion. In using a retrovirus as

vector, the gene of interest is cloned into a virus that lacks most viral genes and is expressed under the control of strong viral promoter sequences. The genetic material of retroviruses is RNA, so that if they infect cells, their RNA genomes are converted to a DNA form (by the viral enzyme reverse transcriptase). The viral DNA is efficiently integrated into the host genome, in which it permanently resides, replicating with the host DNA every cell cycle. Retroviruses can infect virtually every type of mammalian cell, making them very versatile.

### LIBRARIES

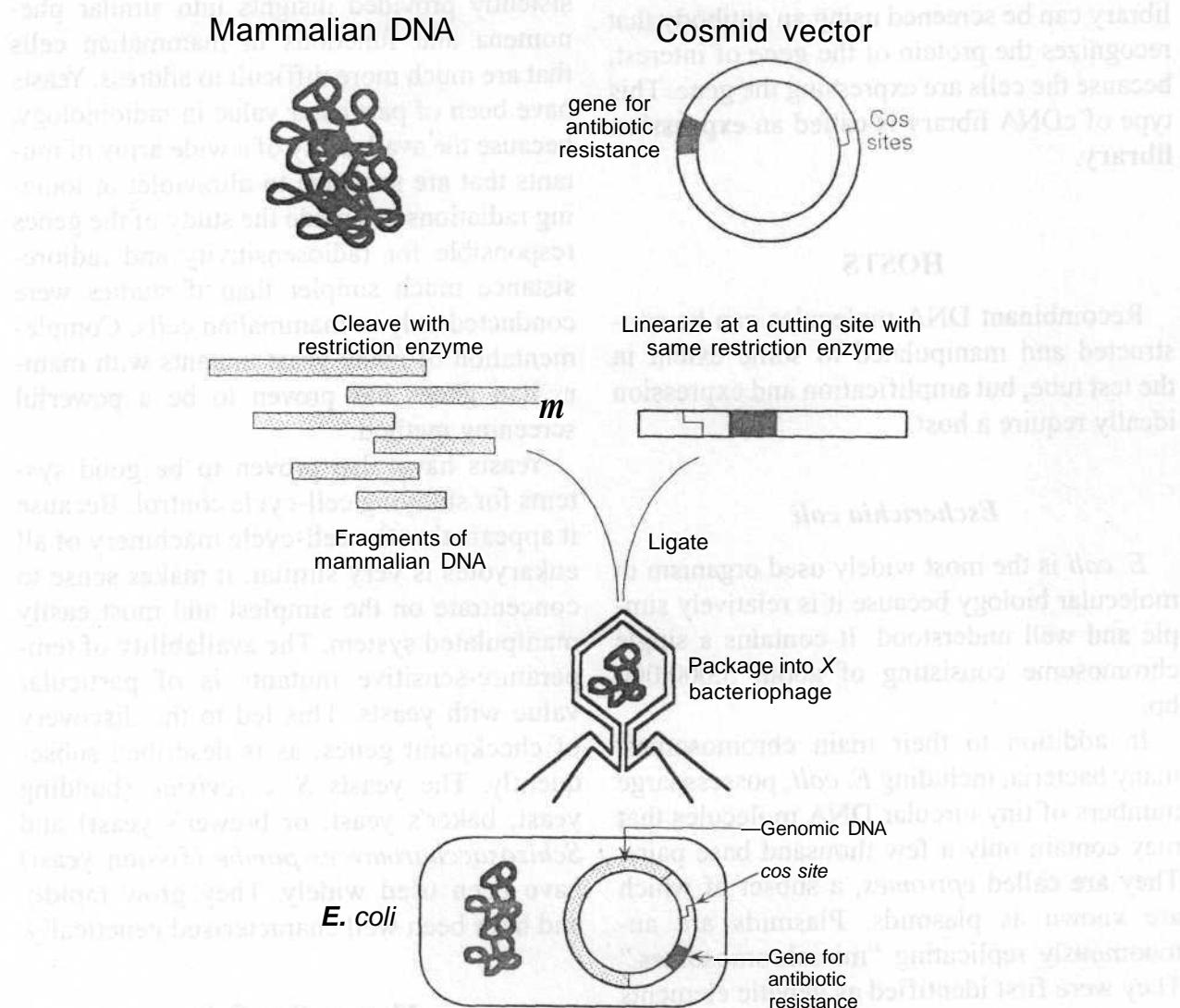
#### Genomic Library

A **genomic library** is a compilation of DNA fragments that make up the entire genome.

Making a genomic library is frequently the starting point of a gene-isolation experiment. DNA is extracted from a tissue sample, or from cultured cells, and a partial digest is made using *EcoRI*, for example. This enzyme has a six-nucleotide recognition sequence, so if the digest is complete, it cuts the DNA into pieces about 4,000 bp long. By reducing the enzyme concentration and incubation time, a partial digest is obtained, so that the *EcoRI* enzyme cuts at only about one in five restriction sites, resulting in fragments of about 40,000 bp.

The genomic DNA fragments then are ligated into a cosmic (or other suitable vector) and "packaged" inside infective bacterio-

phage particles. The assembled bacteriophage particles are used to infect *E. coli* cells, which are spread on plates and incubated in growth medium containing the appropriate antibiotic (e.g., ampicillin), so that only bacteria that have taken up the cosmid survive and grow into colonies. Each colony contains millions of copies of a single genomic DNA insert. About 75,000 colonies encompass the entire genome. If the equivalent of several genomes worth of colonies are screened, then one or more should contain the gene of interest. The trick is to identify that particular colony out of hundreds of thousands. The process is illustrated in Figure 16.8.



**Figure 16.8.** Illustration of the construction of a genomic DNA library. The genomic DNA is digested partially with a restriction enzyme, to produce DNA fragments with an average size of about 40,000 base pairs. These fragments are inserted into cosmids that have a cos site and markers for drug resistance to allow subsequent selection. The cosmids are packaged into bacteriophage, which in turn are used to infect *Escherichia coli*. In this way the DNA fragments are amplified.

### cDNA Library

As an alternative to making a genomic DNA library, it is sometimes more useful to make a cDNA library. **cDNA** is DNA that is complementary to the mRNA and therefore includes only the expressed genes of a particular cell. For eukaryotic cells, the mRNA is usually much shorter than the total size of the gene, because the coding sequences in the genome are split into exons separated by non-coding regions of DNA called *introns*.

cDNA libraries are made in either plasmids or bacteriophage X. Often these vectors have been modified such that the cDNA can be transcribed into mRNA and then translated into protein. If this type of vector is used, the library can be screened using an antibody that recognizes the protein of the gene of interest, because the cells are expressing the gene. This type of cDNA library is called an **expression library**.

### HOSTS

Recombinant DNA molecules can be constructed and manipulated to some extent in the test tube, but amplification and expression ideally require a host.

#### *Escherichia coli*

*E. coli* is the most widely used organism in molecular biology because it is relatively simple and well understood. It contains a single chromosome consisting of about 5,000,000 bp.

In addition to their main chromosomes, many bacteria, including *E. coli*, possess large numbers of tiny circular DNA molecules that may contain only a few thousand base pairs. They are called **episomes**, a subset of which are known as plasmids. Plasmids are autonomously replicating "minichromosomes." They were first identified as genetic elements separate from the main chromosome and carrying genes that conveyed resistance to antibiotics. Foreign DNA can be introduced readily into *E. coli* in the form of plasmids.

Because the DNA of all organisms is made of identical subunits, *E. coli* accepts foreign DNA from any organism. The DNA of bacteria, Drosophila, plants, and humans consist of the same four nucleotides: adenine, cytosine, guanine, and thymine. A foreign gene inside *E. coli* is replicated in essentially the same way as its own DNA.

#### Yeast

Yeasts are simple eukaryotes that have many characteristics in common with mammalian cells but can be grown almost as quickly and inexpensively as bacteria.

The study of yeasts has frequently and consistently provided insights into similar phenomena and functions in mammalian cells that are much more difficult to address. Yeasts have been of particular value in radiobiology, because the availability of a wide array of mutants that are sensitive to ultraviolet or ionizing radiations has made the study of the genes responsible for radiosensitivity and radioresistance much simpler than if studies were conducted only in mammalian cells. Complementation of many yeast mutants with mammalian genes has proven to be a powerful screening method.

Yeasts have also proven to be good systems for studying cell-cycle control. Because it appears that the cell-cycle machinery of all eukaryotes is very similar, it makes sense to concentrate on the simplest and most easily manipulated system. The availability of temperature-sensitive mutants is of particular value with yeasts. This led to the discovery of checkpoint genes, as is described subsequently. The yeasts *S. cerevisiae* (budding yeast, baker's yeast, or brewer's yeast) and *Schizosaccharomyces pombe* (fission yeast) have been used widely. They grow rapidly and have been well characterized genetically.

#### Mammalian Cells

The limited number of cell systems used in radiation and chemical transformation studies can be separated broadly into two categories.

The first category includes short-term explants of cells derived from rodent or human embryos with a limited life-span. These include:

Hamster embryo cells  
Rat embryo cells  
Human skin fibroblasts  
Human foreskin cells  
Human embryo cells

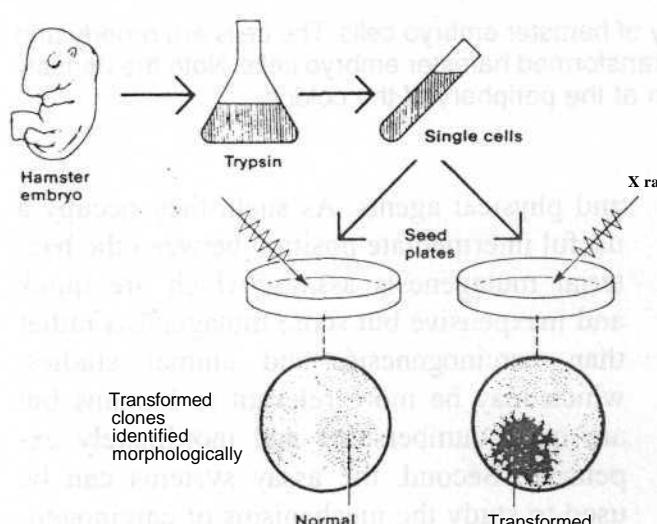
These cell assay systems can be used to assess the expression or activity of foreign genes transfected into them, or they may be used in studies of oncogenic transformation induced by radiation or chemicals.

In practice, the bulk of the experimental work has been performed with hamster or rat embryo cells. One advantage of such systems is that they consist of diploid cells, so that parallel cytogenetic experiments can be performed. Cell survival and cell transformation can be scored simultaneously in the same dishes.

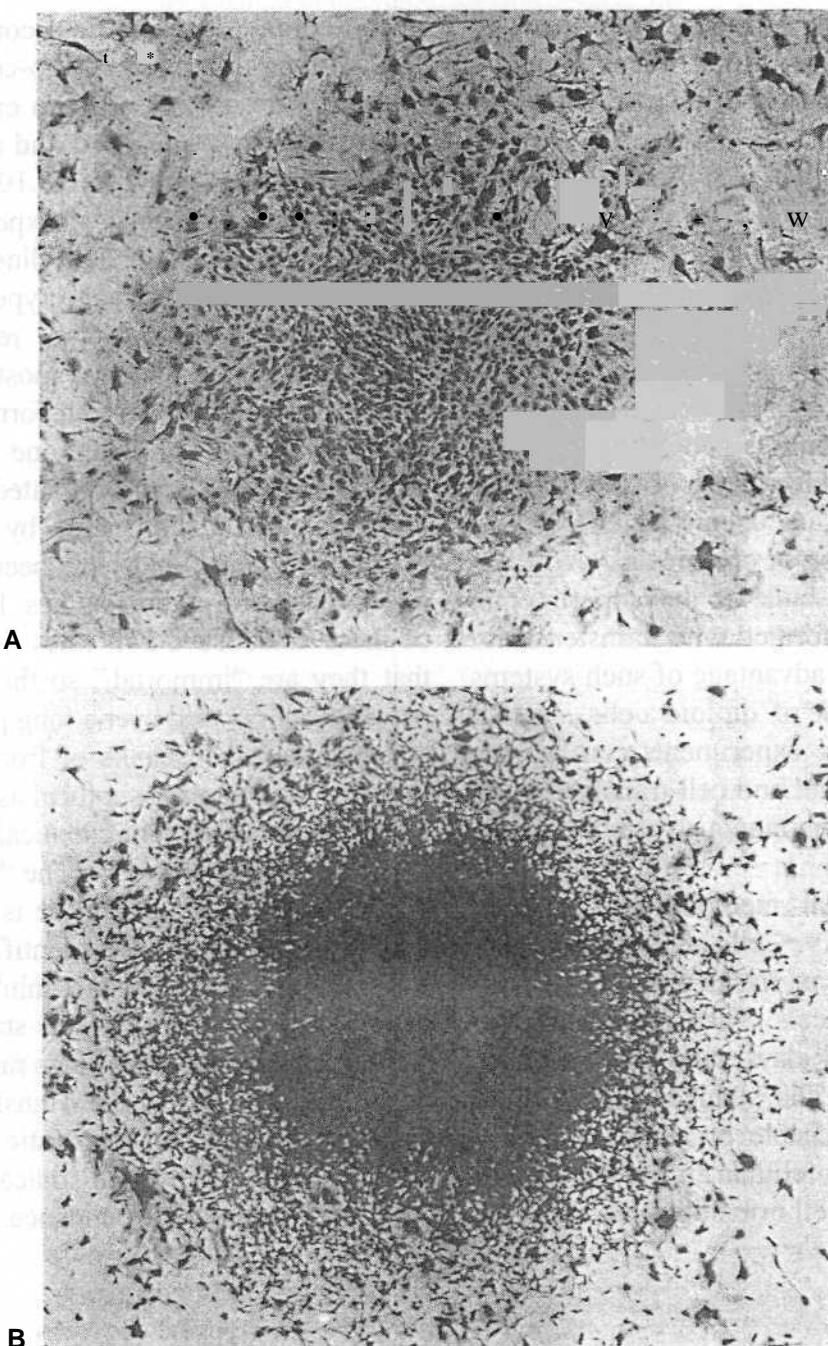
The experimental methodology is illustrated in Figure 16.9. Cells are seeded at low density into dishes or flasks, and treated with radiation or chemicals. They are allowed to grow for 8 to 10 days, and the resultant colonies are fixed and stained. Transformed colonies are identifiable by dense multilayered cells, random cellular arrangement, and haphazard cell-to-cell orientation accentuated

at the colony edge. Normal counterparts are flat, with an organized cell-to-cell orientation and no piling up of cells. An example of the contrast between a normal and a transformed colony is shown in Figure 16.10.

The second category of experimental systems includes established cell lines that have an unlimited lifespan. The karyotype of these cells shows various chromosomal rearrangements and heteroploidy. The two most widely used established cell lines for transformation studies are the BALB/C-3T3 cell line and the C<sub>3</sub>H 10T1/2 cell line. Both originated from mouse embryos, are transformable by a variety of oncogenic agents, and have been used extensively in transformation studies. The advantage of these established cell lines lies in the fact that they are "immortal," so that a particular passage can be used over a long period of time and maintained in banks of frozen cells. The transformation assay is a focal assay. Cells are treated with radiation or chemicals and then allowed to grow for 6 weeks. The "**normal**" cells stop growing after confluence is reached, and transformed foci can be identified against a background of the contact-inhibited normal cells because they are densely stained, tend to pile up, and show a criss-cross random pattern at the edge of the focus. Transformed cells, identified by their characteristic morphology, grow in soft agar, which indicates that they have lost anchorage dependence, and produce



**Figure 16.9.** Protocol for the assay of oncogenic transformation in hamster embryo cells by radiation. Midterm hamster embryos are removed, minced, enzymatically dissociated, and seeded as single cells on feeder layers. They then are treated with either radiation or chemicals, and the resultant colonies (normal and **transformed**) are scored after 8 to 10 days of incubation.

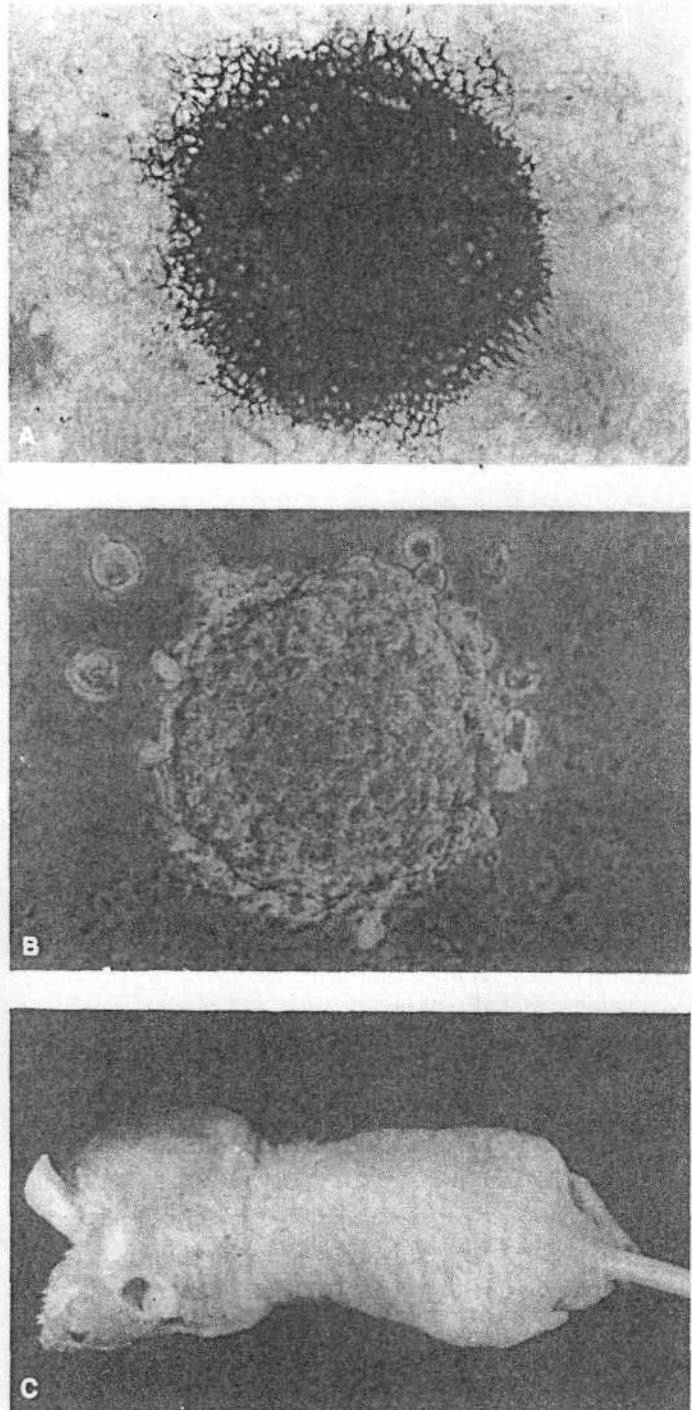


**Figure 16.10.** A: A normal untransformed colony of hamster embryo cells. The cells are orderly and show contact inhibition. B: A colony of radiation-transformed hamster embryo cells. Note the densely stained, piled up cells and the criss-cross pattern at the periphery of the colony.

fibrosarcomas if injected into suitably prepared animals. This is illustrated in Figure 16.11.

The *in vitro* assay systems based on mammalian cells have two quite different uses in radiobiology. First, they may be used to accumulate data and information that are essentially pragmatic in nature; for example, they may be used to compare and contrast the oncogenic potential of a variety of chemical

and physical agents. As such, they occupy a useful intermediate position between the bacterial mutagenesis assays, which are quick and inexpensive but score mutagenesis rather than carcinogenesis, and animal studies, which may be more relevant to humans but are quite cumbersome and inordinately expensive. Second, the assay systems can be used to study the mechanisms of carcinogen-



**Figure 16.11.** A: A type III transformed focus of  $C_3H\ 10T1/2$  cells induced by the hypoxic cell sensitizer etanidazole (SR 2508). Note the multilayered growth and the criss-crossing of cells at the periphery of the clone over a contact-inhibited background of nontransformed cells. B: Cells from the above focus were plucked, expanded in culture, and plated into semi-solid medium; they formed colonies, indicating that they had lost anchorage dependence. This is an indication of malignancy. C: The ultimate test of malignancy is whether cells from a type III transformed clone injected into a suitably prepared animal produce a tumor (a fibrosarcoma) that eventually kills the animal.

esis. In this context, transformation assays have played a vital role in unfolding the oncogene story, because transfecting DNA from human tumors into one or the other of the established cell lines used for transformation, most often 3T3 or rat-2 cells, and observing the induction of transformed foci is one way to detect the expression of an oncogene, as illustrated in Figure 16.12.

### DNA-mediated Gene Transfer

Gene transfer is now a routine tool for studying gene structure and function. Gene transfer into mammalian cells is an inefficient process, so that an abundant source of starting cells is necessary to generate a workable number of transfected cells, that is, cells containing a transferred gene.

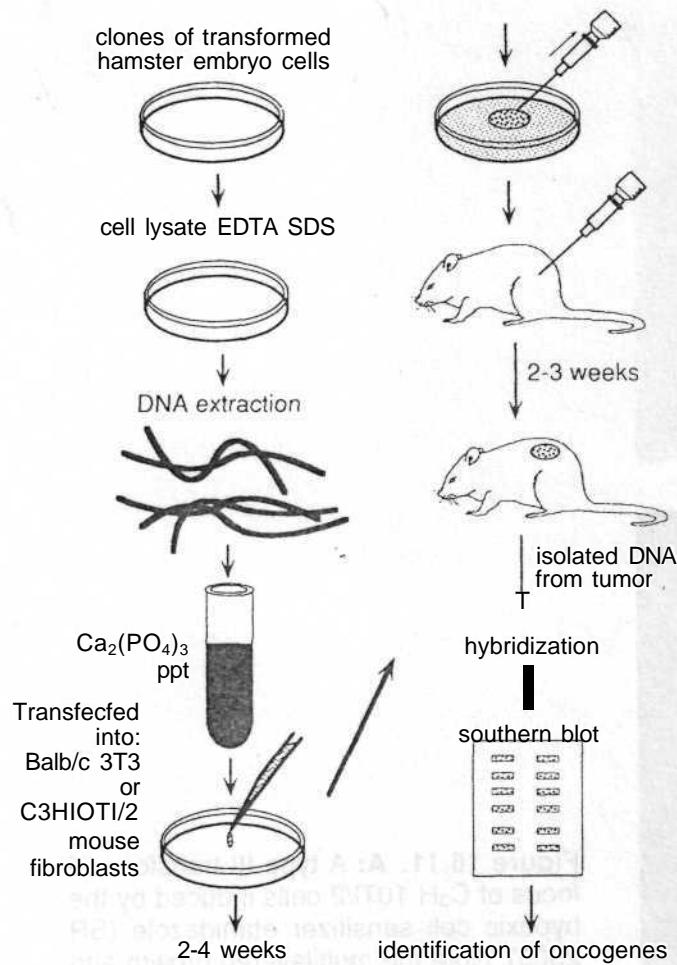


Figure 16.12. Schematic diagram of a typical DNA transfection protocol, in which oncogenes can be isolated from cells transformed *in vitro* by either radiation or chemical carcinogens. DNA sequences then are characterized using a Southern blot hybridization.

Mammalian cells do not take up foreign DNA naturally; indeed, they try to protect themselves from invading DNA. Consequently, one of several tricks must be used to bypass natural barriers.

1. Microinjection: This is the most direct, but most difficult procedure to accomplish. DNA can be injected, cell by cell, directly into the nucleus through a fine glass needle.
2. Calcium phosphate precipitation: Cells take up DNA relatively efficiently in the form of a precipitate with calcium phosphate. The efficiency varies markedly from one cell line to another. For example, NIH 3T3 cells are particularly receptive to foreign DNA introduced by this technique. This is the most widely used method of gene transfer and is illustrated in Figure 16.13. High molecular weight

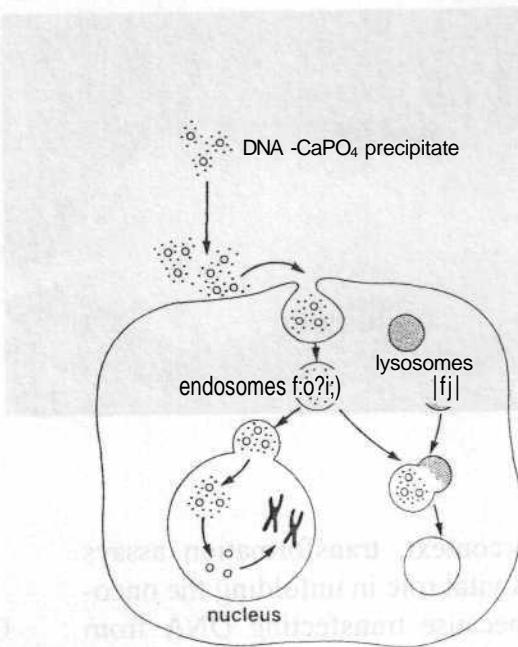


Figure 16.13. The technique of transfection of DNA from a mammalian cell, cut into fragments, can be introduced into another cell as part of calcium phosphate precipitate. The donor DNA is integrated at low frequency into the genome of the recipient cell.

DNA is mixed with insoluble calcium phosphate as a carrier and layered onto cells in petri dishes. Typically, a plasmid containing a selectable marker, such as G418 resistance, is copipetted and cotransfected into cells. In this way cells that take up DNA can be selected. Of the cells that take up DNA, only a small percentage ultimately integrate the DNA into their genomes (stably transfected). If a fragment of DNA containing an activated oncogene is transfected into NIH 3T3 cells, morphologic transformation of the cell occurs, leading to loss of contact inhibition, and the cells produce tumors if injected into immune-suppressed animals.

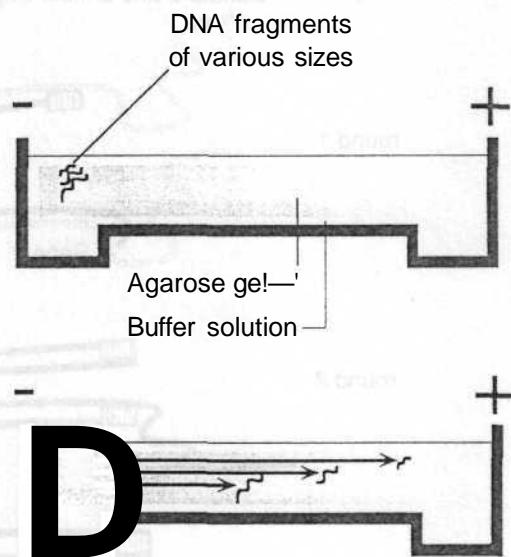
3. **Electroporation:** This technique is useful for cells that are resistant to transfection by calcium phosphate precipitation. Cells in solution are subjected to a brief electrical pulse that causes holes to open transiently in the membrane, allowing foreign DNA to enter.
4. **Viral vectors:** The ultimate means of transfection involves the use of a retrovirus—this is essentially the analogue of using bacteriophage to get DNA into bacteria. The genetic material of a retrovirus is RNA, so that when retroviruses infect mammalian cells their RNA genomes are converted to DNA by the viral enzyme **reverse transcriptase**. The viral DNA is incorporated efficiently into the host genome, replicating along with the host DNA at each cell cycle. If a foreign gene is incorporated into the retrovirus, it is permanently maintained in the infected mammalian cell. **Oncogenes**, genes that can cause cancer, and their counterpart, **tumor-suppressor** genes, can be studied by incorporating them into retroviral vectors.

## AGAROSE GEL ELECTROPHORESIS

The purpose of agarose gel electrophoresis is to separate pieces of DNA of different size. This technique is based on the fact that DNA

is negatively charged. Under the influence of an electric field, DNA molecules move from negative to positive poles and are sorted by size in the gel. In a given time, small fragments migrate through the gel farther than large fragments.

The technique, illustrated in Figure 16.14, is as follows: Molten agarose is poured into a tray in which a plastic comb is suspended near one end to form wells in the gel after it has solidified like gelatin. The concentration of the agarose is varied according to the size of the DNA fragment to be separated and visualized: high concentration for small fragments, lower concentration for larger fragments. The solidified gel is immersed in a tray containing an electrolyte to conduct electricity. The DNA samples, mixed with sucrose and a visible dye, are pipetted into the wells, and the electric field is connected. Electrophoresis is monitored by observing the movement of the dye in the electric field. After separation is



Small DNA fragments travel further than large

**Figure 16.14.** Illustrating agarose gel electrophoresis, DNA is negatively charged, so that under the influence of an electric field it migrates toward the anode. During electrophoresis, DNA fragments sort by size, small molecules moving farther than larger molecules. Because smaller molecules move farther than larger molecules in a given time, polyacrylamide gel electrophoresis often is employed to separate smaller DNA fragments.

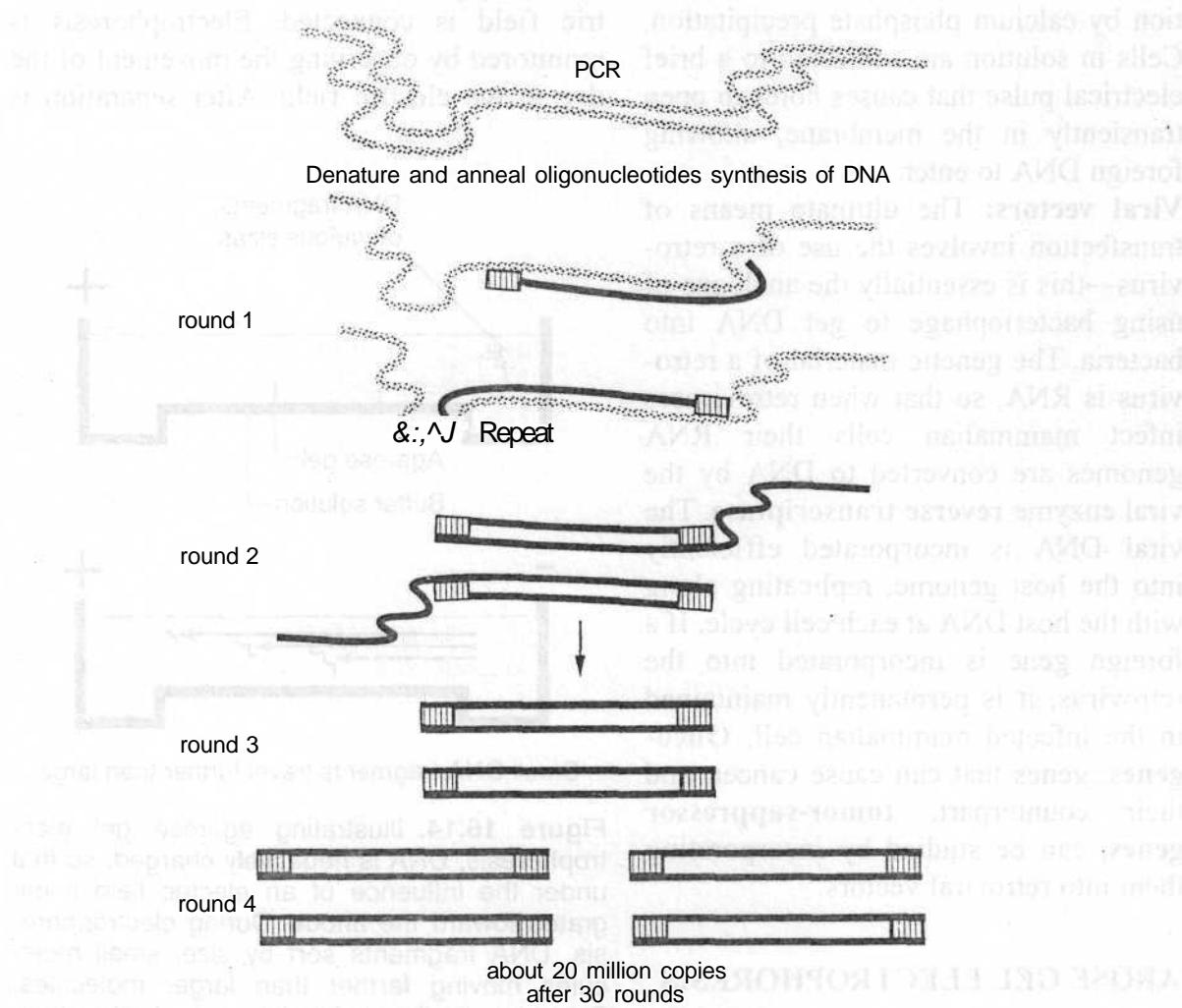
complete, the gel is soaked in ethidium bromide, which intercalates into DNA and fluoresces under ultraviolet light to make the position of the DNA visible. Several examples are shown elsewhere in this chapter.

## POLYMERASE CHAIN REACTION

The polymerase chain reaction (PCR) technique uses enzymatic amplification to increase the number of copies of a DNA fragment of up to about 6,000 bp. The principle is based on primer extension by DNA polymerases, which was discovered in the 1960s. First, primers, which are complementary to the 5' end of the double-stranded DNA se-

quence to be amplified, are synthesized. The two primers are mixed in excess with a sample of DNA that includes the fragments to be amplified, together with a heat-stable Taq DNA polymerase from *Thermits aquaticus*, a bacterium that inhabits hot springs. The four deoxyribonucleotide triphosphates also are provided in excess; one or more of them may be radioactively labeled. The power of the technique is that it can be used to amplify a DNA fragment from total genomic DNA. The PCR technique is illustrated in Figure 16.15.

The amount of the sequence is doubled in each cycle, which takes about 7 minutes. During each cycle the sample is heated to about 94°C to denature the DNA strands, then



**Figure 16.15.** The polymerase chain reaction for the amplification of DNA fragments. The number of DNA molecules is doubled in each cycle, which takes about 7 minutes, so that in a matter of several hours, millions of copies of a DNA fragment can be made. (Courtesy of Dr. Greg Freyer.)

cooled to about 50°C to allow the primers to anneal to the template DNA, and then heated to 72°C, the optimal temperature for Taq polymerase activity. In a matter of a few hours, a million copies of the DNA fragment can be obtained in an essentially automated device. PCR has found many applications in both basic research and clinical settings. For example, it has been used to detect malignant cells in patients with leukemias that are characterized by consistent translocation breakpoints. Primers that span the breakpoint are added to a bone-marrow sample and subjected to multiple cycles of PCR. Even one cell in a million with the translocation can be detected.

### GENE-CLONING STRATEGIES

In the most general terms, there are three steps involved in cloning a gene:

1. Choose a source of DNA, which may be genomic DNA or cDNA.
2. Construct a library, which is a collection of DNA fragments inserted into an appropriate vector.
3. Screen the library to locate the gene of interest.

The first two steps already have been described. There are four principle methods to screen for a gene.

#### Functional Complementation

This technique depends on the DNA segment producing its corresponding protein within the cell, thereby giving the host a specific and detectable phenotype. For example, in screening for a repair gene that confers resistance to radiation or to a particular chemical agent (*e.g.*, mitomycin C), a sensitive line of cells that lacks the functional gene is transfected with a library of genomic DNA from cells that contain the active gene.

Only one in many thousands of cells takes up the gene of interest, but these cells acquire resistance and can be selected by long-term treatment with the cytotoxic agent (*e.g.*, **st-rays**, mitomycin C).

During DNA transfection many genes enter the cell, so it is necessary to first determine which of the introduced sequences contain the repair gene. The DNA fragment from the library can be identified by its association with the vector sequence. If this is a cosmid, the incoming DNA can be rescued from total genomic DNA. Once all of the library sequences are isolated they must be screened individually to determine which contains the repair gene or genes. This can be done by reintroducing the purified vector genomic DNA clones into the sensitive cell line to determine which clone confers resistance.

#### Hybridization

Double-stranded DNA can be denatured; that is, the hydrogen bonds between the base pairs can be disrupted, causing the complementary strands to disassemble. DNA denatures under a variety of conditions, such as in the presence of high pH or high temperature. Under the right conditions, the two single-stranded molecules can re-form the original duplex DNA molecule. This process of complementary single-stranded molecules lining up to form a double-stranded molecule is known as hybridization. Under "low stringency" conditions, partial hybridization takes place if the strands have a lesser degree of complementarity.

A genomic library, prepared as described previously, can be screened for the presence of a particular gene of interest by hybridization. The library consisting of bacteriophage or cosmids is grown on plates. A replica copy of each plate is made by transferring the colonies or plaques onto a filter disk, rather like a rubber stamp. The cells or bacteriophage are lysed and their DNA denatured, followed by screening with a probe that has a sequence complementary to the gene of interest. The probe is labeled **with a** ( $\beta$ -emitting radionuclide (such as phosphorus-32) so that it can be detected easily. The filters are incubated under conditions that favor hybridization of the probe to its complementary sequence (neutral pH, presence of sodium ions to neutralize the negative

charge on the DNA, and an elevated temperature). After removing the unhybridized probe, x-ray film is pressed tightly against the filters so that  $\beta$ -particles emitted from the phosphorus-32 expose the film; this is called **autoradiography**. After development, the exposed areas appear as black spots on the film, corresponding to the plaques/colonies that contain the gene of interest.

### Oligonucleotide Probes

If it is possible to obtain a partial or complete amino acid sequence of the protein encoded by the gene under study, the coding sequence derived from these amino acids can be used to synthesize an oligonucleotide, a DNA sequence of a few nucleotides. This can be used as a probe for the gene of interest. A six-amino-acid sequence can be used to derive a series of 18 nucleotide DNA probes that take into consideration the redundancy of the genetic code. This mixture of oligonucleotides is labeled with a  $\beta$ -emitting radionuclide so that the plaques or lysed bacterial colonies to which it hybridizes in the DNA library can be identified readily by autoradiography.

### Antibody Probes

**Antibodies** are formed as part of the immune response of animals to the presence of a foreign substance (an antigen).

To make antibodies against a specific protein, the foreign protein is used to immunize a laboratory mouse. B lymphocytes from the animal's spleen are fused with myeloma cells (derived from a mouse bone cancer) to produce hybridomas that are essentially immortal. Individual hybridomas are isolated that produce a monoclonal antibody. This technique is used only to screen a cDNA **expression library**.

### Positional Cloning

**Positional cloning** is a strategy used to isolate a gene if no information is available about its protein product. This is the situation for many human inherited disorders in which the

underlying biochemical defects are simply unknown.

The identification of genes responsible for inherited human disorders is an integral part of the Human Genome Project. Several approaches are combined to isolate genes by positional cloning. As its name implies, the first step in this procedure is to determine the chromosomal location of the gene. This information is used to clone DNA sequences from that location, and these sequences are used as probes to find the gene itself. Linkage analysis facilitates the localization of a gene by comparing within a family the inheritance of a mutant gene with the inheritance of DNA markers of known chromosomal location. This approach requires identification of several markers and an increase of the markers within the defined region of the chromosome. Coinheritance of the disease gene and the marker suggests that they are physically close together on the chromosome. This *linked DNA sequence* is used as the starting point to "walk" or "jump" to the gene by cloning DNA fragments that are even more tightly linked and therefore closer to the gene.

After the gene has been isolated, the DNA sequence can be determined and analyzed to predict the biochemical properties of the encoded protein. The technique of positional cloning has become a familiar component of modern human genetics research. After a halting start in the mid-1980s, the number of disease genes isolated by cloning efforts based solely on pinpointing their position in the genome is growing rapidly. More than 110 genes have been identified so far. The positional candidate approach, which combines knowledge of map position with the increasingly dense human transcript map, greatly expedites the search process and has become the predominant method of disease-gene discovery.

It is important to recognize the difference between positional cloning and functional complementation. Oncogenes were cloned and sequenced before tumor-suppressor genes because they act in a dominant fashion and so can be detected by functional complemen-

tion, which is by far the simpler procedure. Tumor-suppressor genes at a cellular level are recessive-acting and so are not so easily detected by complementation; most of those discovered so far have been isolated by positional cloning. The other limitation of functional complementation becomes evident in the search for a very large gene. If the gene is so large that it is never contained in any of the DNA fragments used to complement, then the function of the gene is not recovered and thus the approach fails to identify the gene. An example is the cloned gene for ataxia telangiectasia, termed the ATM (A-T mutated) gene. This gene has 66 exons and is 150 kb in

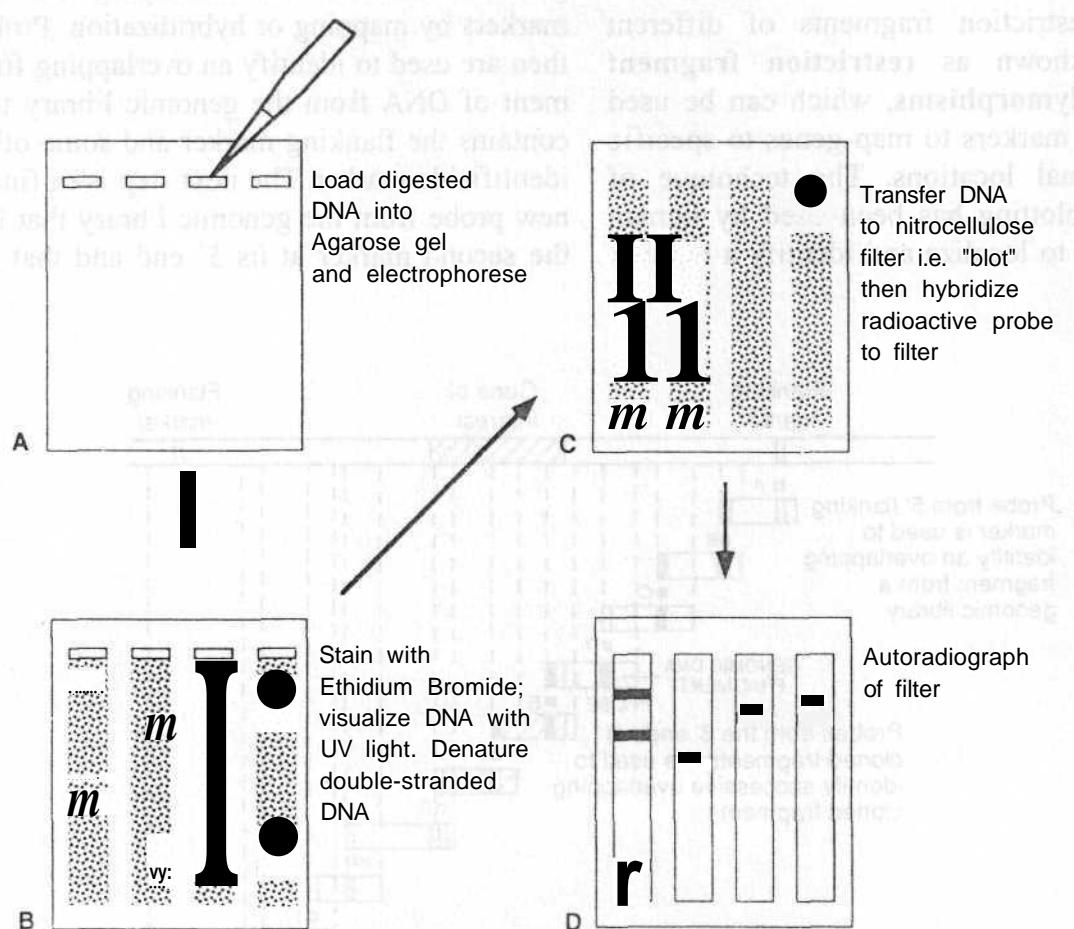
length; it was finally cloned by the positional cloning approach in 1995, after attempts by other means had failed for quite a few years.

## GENE ANALYSES

### Mapping

#### *Southern Blotting*

Southern blotting, named after its discoverer Ed Southern, can be used to study a gene without cloning it. As in library screening, it is based on hybridization. The technique is illustrated in Figure 16.16.



**Figure 16.16.** The technique of Southern blot analysis. A: Digested DNA fragments are loaded into the wells of an agarose gel and subjected to electrophoresis. B: DNA fragments move different distances according to their sizes and can be visualized under ultraviolet illumination after staining with ethidium bromide. C: The DNA is denatured and then transferred to a nitrocellulose filter by capillary action; that is, a "blot" is made. A probe labeled with a radionuclide is hybridized to the filter. D: An autoradiograph is made of the filter. Bands corresponding to DNA strands to which the probe hybridize are visualized clearly.

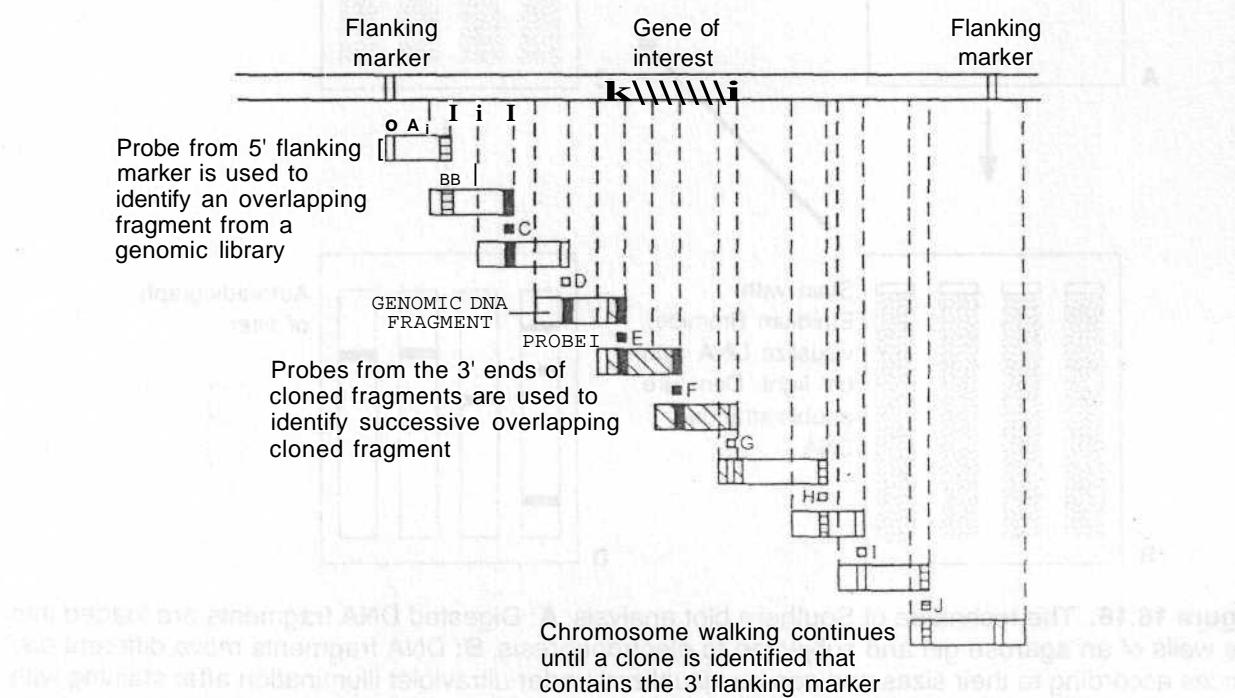
DNA is extracted from a tissue or cell culture of interest, digested with one or more restriction enzymes, and electrophoresed on an agarose gel. The duplex DNA is denatured and transferred to a nitrocellulose filter, a technique called *blotting*. The filter then is bathed in a solution containing a specific probe labeled with phosphorus-32 under hybridization conditions. The radioactive probe hybridizes only to its complementary sequence, and this can be identified by sandwiching the filter against an x-ray film. Exposed regions on the film appearing as dark bands indicate the positions of this sequence on the gel.

Southern blotting is useful for analyzing the sizes of DNA fragments lost in radiation-induced mutations. It is also useful for detecting structural variations in DNA that result in restriction fragments of different lengths, known as **restriction fragment length polymorphisms**, which can be used as genetic markers to map genes to specific chromosomal locations. The technique of Southern blotting has been used by human geneticists to localize and identify a number

of disease genes and also has been used in "DNA fingerprinting."

### Chromosome Walking

The chromosome-walking technique is necessary if a gene of interest has been mapped to a specific arm of a chromosome and one wants to isolate the gene. It is not needed if the gene of interest is already contained in a discrete DNA fragment obtained from a DNA library. The process is illustrated in Figure 16.17. The starting point is to identify a piece of DNA, a flanking marker, that is close to the gene. This is accomplished by making a genomic library of large DNA fragments (20,000-40,000 bp) that includes the gene of interest and identifying flanking markers by mapping or hybridization. Probes then are used to identify an overlapping fragment of DNA from the genomic library that contains the flanking marker and some other identifiable marker. The next step is to find a new probe from the genomic library that has the second marker at its 5' end and that in-



**Figure 16.17.** Illustration of the technique of chromosome walking. Working from a flanking DNA marker, overlapping clones are successively identified that span a chromosomal region containing a gene of interest. (Courtesy of Dr. Greg Freyer.)

eludes yet another identifiable marker (C). This process of identifying overlapping probes that span a chromosomal region containing a gene of interest is referred to as chromosome walking and is continued until the gene of interest is reached.

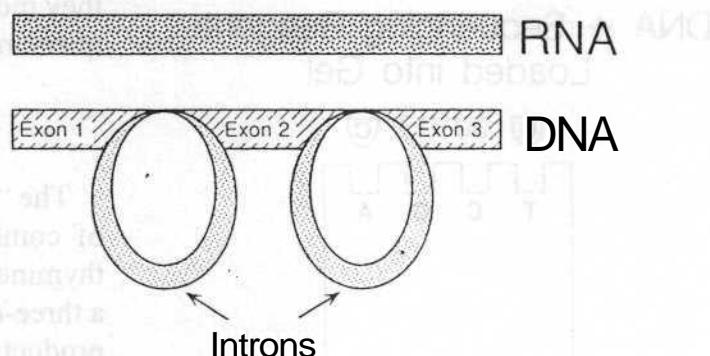
### Contiguous Mapping

**Mapping** refers to the determination of the physical location of a gene or genetic marker on a chromosome. **Contiguous mapping** refers to the alignment of sequence data from large, adjacent regions of the genome to produce a continuous nucleotide sequence of a region of a chromosome. The basic idea is to orient physical markers, such as restriction fragment length polymorphisms, on adjacent fragments so that they can be lined up and the nucleotide sequence can be made continuous. If, for example, restriction fragments from a DNA library are sequenced, relating these sequences to known physical markers eventually can produce the nucleotide sequence of the entire genome. This is the goal of the Human Genome Project, but the task is so massive that it cannot be accomplished without the development of automated sequencing technology and sophisticated computer strategies to store and handle the data.

### DNA Sequence Analyses

#### Introns and Exons

It is at once obvious from a comparison of a mature cytoplasmic mRNA transcript with its parental DNA that the mRNA sequence is not contiguous with the DNA sequence. Some blocks of DNA sequence are represented in the mRNA, others are not. DNA is transcribed into pre-mRNA. During this process of splicing, large regions called *introns* are removed and the remaining *exons* are joined together. Almost all genes from higher eukaryotes contain introns; genes may have only a few or as many as 100 introns. Typically, introns make up the bulk of the gene.. For example, in the gene involved with muscular dystrophy, the mRNA consists of 14,000 bases, whereas the



**Figure 16.18.** DNA is made up of nucleotide sequences that are transcribed to mRNA, called *exons*, and sequences that are excised from pre-mRNA during RNA processing, called *introns*.

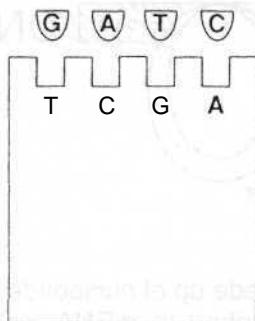
gene spans more than 2 million bp. The way in which exons are transcribed and introns omitted is illustrated in Figure 16.18.

#### Sequencing Gel: Chain-termination Method

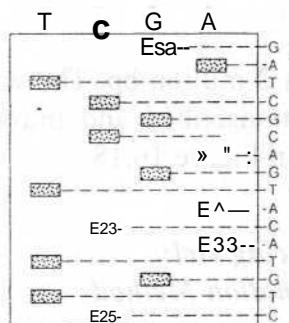
This technique depends on two characteristics of DNA synthesis:

1. With the availability of the four deoxyribonucleotide triphosphates, dATP, dTTP, dCTP, and dGTP, synthesis of a new strand of DNA is initiated by DNA polymerase at any point at which a short DNA primer hybridizes to a **single-stranded** DNA template.
2. If the deoxyribonucleotide triphosphates are mixed with *di* deoxyribonucleotide triphosphates, the DNA chain elongation ceases if a *di* deoxyribonucleotide triphosphate is incorporated. The preparation of a sequencing gel is shown in Figure 16.19. Four reaction tubes are set up, each containing, in addition to the DNA template to be sequenced, a primer sequence, DNA polymerase, and all four deoxyribonucleotide triphosphates (dATP, dTTP, dCTP, and dGTP), one of which is labeled with the radionuclide phosphorus-32. One *di* deoxyribonucleotide triphosphate is added to each of the tubes. The concentration of the *di* deoxyribonucleotide triphosphate is such that it is incorporated in only about 1

## DNA + Sequencing Reagents Loaded into Gel



## Autoradiograph of Gel



**Figure 16.19.** Illustration of a sequencing gel based on the chain-termination method.

in 100 nucleotides, thereby stopping the synthesis of the strand. In the reaction containing *tMATP*, DNA fragments are made of all lengths terminating in adenine; in the reaction containing *didCTP*, all fragment lengths are made terminating with cytosine.

After the reactions are completed the newly synthesized DNA fragments are separated on a polyacrylamide gel and an autoradiograph is made from the gel. Because the DNA fragments were of all different lengths, they have moved different distances under the influence of the electric field. The sequence then can be read, starting at the bottom. An actual gel is shown elsewhere in this chapter to illustrate one of the examples of the use of molecular techniques in radiobiology.

DNA sequencing has been automated as part of the Human Genome Project. With the appropriate equipment, fluorescein-labeled primers are used that can be monitored as

they move past a detector at the end of the sequencing gel.

## Deduced Protein Sequences

The "message" in DNA is linear, consisting of combinations of the four bases adenine, thymine, guanine, and cytosine. Proteins have a three-dimensional form. The first step in the production of a protein from the message in the DNA is that the DNA code is **transcribed** in the nucleus into pre-mRNA, which is a complementary version of the DNA code. Next, the pre-mRNA is processed to mRNA by removal of its introns. The mRNA enters the cytoplasm, where it associates with a **ribosome** and the mRNA message is **translated** into "chains" of amino acids. The steps from DNA to protein are illustrated in Figure 16.3. The genetic code is read as triplets. Thus, three nucleotides code for an amino acid. There are 64 possible combinations of the four bases; 61 of these code for the 20 amino acids. Consequently, there is degeneracy, or redundancy, in the code, because the same amino acid is coded for by more than one triplet. Nearly all proteins begin with the amino acid methionine, for which the codon is AUG. Consequently, this represents the "start" signal for protein synthesis. The "stop" signal is any one of the three codons for which there is no naturally occurring tRNA, namely UAA, UAG, or UGA. Most proteins contain only one polypeptide chain, characterized by a unique sequence of amino acids. There are, however, other proteins formed through the aggregation of separately synthesized chains that have different sequences.

Because the message in the DNA results in a unique protein, it is clearly possible to go in the reverse direction and deduce the sequence of the gene from the proteins expressed by the gene. This is not straightforward, because there is redundancy in the code for specifying amino acids from triplet RNA codons. With four bases and triple codons, far more combinations are available than there are amino acids, and so in practice the same amino acid corresponds to more than one sequence of bases in the mRNA.

There are ways to circumvent this problem, as described in the section on oligonucleotide probes. Once a DNA sequence is obtained, the amino acid sequence of the corresponding protein can be determined.

### ***Homologies to Known Genes and Proteins***

Interpreting a new sequence is no easy matter. An early step is to find an "open reading frame," that is, a sequence that corresponds to a "long" stretch of amino acids and does not contain a stop codon. This is more likely to be part of an active gene, because protein-coding regions are not interrupted by stop codons.

When a new gene is sequenced the information is entered into various databases to search for sequence homology with existing genes. These homologies are important in identifying function and the relationship between genes. Typically, in searching a data base, the coding sequences of both the entire gene and also of the cDNA are entered. The cDNA sequence is often more useful, because intron sequences diverge rapidly, as do gene-flanking sequences, but exon sequences (primarily coding for amino acids) tend to be conserved. It is even more desirable to search for a protein sequence, because changes can occur **in** the DNA sequence that do not lead to changes in the amino acid sequence because of the degeneracy of the genetic code.

### **Polymorphisms or Mutations**

#### ***Restriction Fragment Length Polymorphisms***

Relatively small differences **in** similar DNA sequences, or **polymorphisms** as they are called, may result from point mutations, deletions or insertions, or varying numbers of copies of a DNA fragment (so-called tandem repeats). A Southern blot analysis can be used to detect DNA polymorphisms, using a probe that hybridizes to a polymorphic region of the DNA molecule. Other techniques also may be used to detect polymorphisms.

If a particular restriction enzyme is used to cut human DNA, a polymorphic locus yields

restriction fragments of different sizes. These are restriction fragment length polymorphisms. Deletions, insertions, or tandem repeats involving more than about 30 nucleotides can be detected as recognizable shifts in the Southern blot hybridization pattern. Even a point mutation can be detected if the resultant change in sequence removes or adds a new recognition site at which a restriction endonuclease cuts.

#### ***Single-Stranded Conformation Polymorphism***

Several methods have been developed to screen for an **unknown** mutation in a gene. Among them, single-stranded conformation polymorphism is particularly useful. A single base-pair difference between two short single-stranded DNA molecules (such as the difference between a wild-type gene and one that has suffered a point mutation) results in a difference in conformation between the two strands that, remarkably enough, can be detected by a difference in the molecule's electrophoretic mobilities on a neutral polyacrylamide gel. The same change would go undetected if electrophoresis were carried out under denaturing conditions in which strands separate only according to size, not base composition. This represents a powerful technique to screen for mutations in an oncogene (such as *ras*) or in a tumor-suppressor gene (such as p53).

### **Expression: Northern Blotting and Hybridization**

**In** northern blotting, a probe is hybridized to RNA, in contrast to Southern blotting, in which the probe is hybridized to DNA.

This is the best technique available to monitor mRNA abundance and turnover in cells. The techniques used in northern blotting are essentially similar to those described for Southern blotting except that RNA is used in place of DNA, is already single-stranded, and does not need previous digestion with restriction enzymes. The intensity of the bands on the final autoradiograph reflects the abundance of the "message" and its expression in a particular cell or tissue.

### STUDY OF PROMOTERS: THE CAT ASSAY

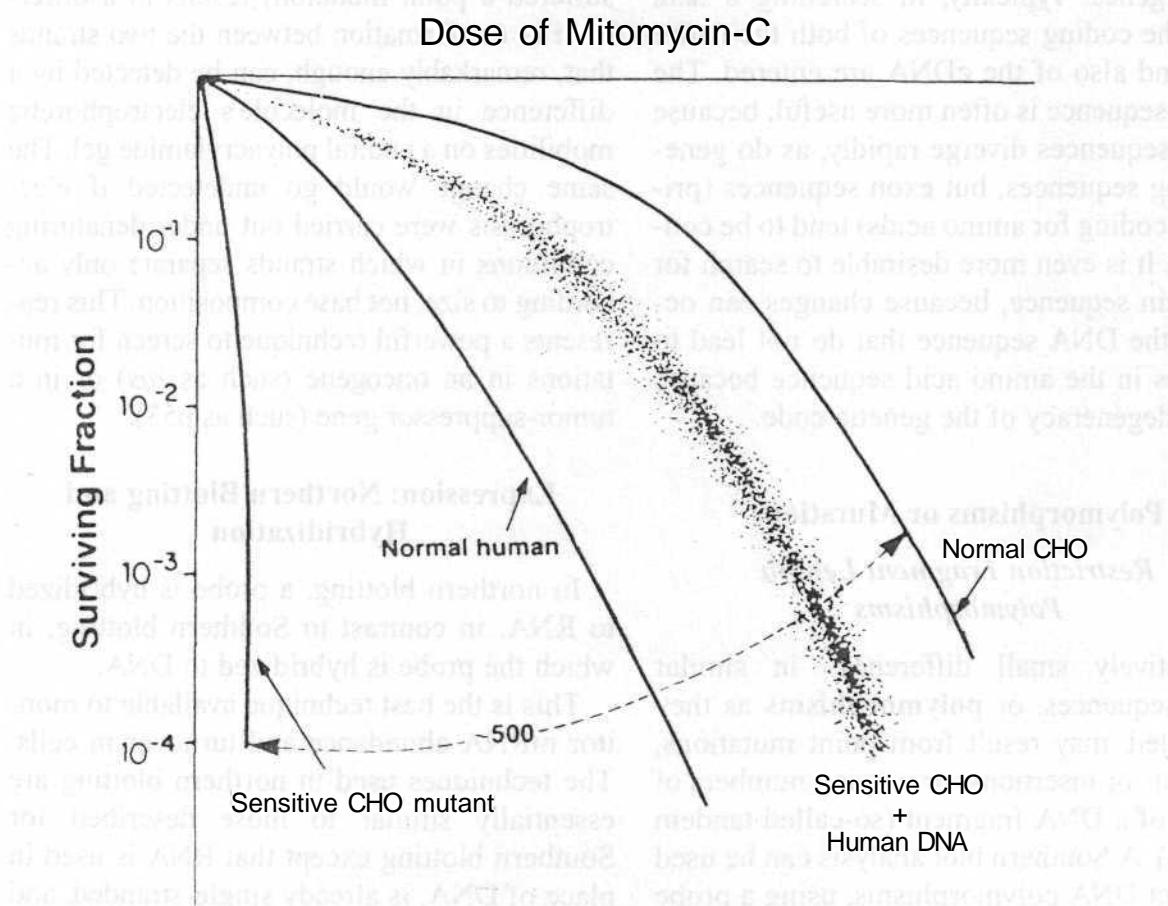
It is frequently of vital interest to know if a promoter is working at all and at what level, in studies for example in which attempts are being made to improve the promoter by altering the nucleotide sequence. The promoter region is upstream (5') of the start of transcription, occupying approximately 100 nucleotides and frequently containing TATA and CCAAT boxes. The straightforward way to proceed would be to measure the RNA or protein produced by the gene of interest, but this may be difficult and time-consuming to accomplish. A shortcut is to use a reporter assay such as the CAT assay. The promoter in question is ligated to the bacterial CAT (chloramphenicol acetyl transferase) gene and introduced into eukary-

otic cells. The CAT gene acetylates chloramphenicol to an extent, depending on the efficiency of the promoter, and this reaction is highly sensitive and easy to detect on a gel.

### EXAMPLES OF PROJECTS OF DIRECT OR INDIRECT INTEREST TO RADIOBIOLOGY EMPLOYING MOLECULAR TECHNIQUES

#### The First Repair Gene in a Mammalian Cell Identified and Cloned by Gene Transfer (Rubin *et al*, 1983; van Duin *et al.*, 1986)

A mutant line of Chinese hamster ovary (CHO) cells was used that was about 500 times as sensitive to killing by mitomycin C as wild-type cells (Fig. 16.20). Total genomic



**Figure 16.20.** Dose-response curves for mitomycin C for wild-type Chinese hamster ovary (CHO) cells, for a very sensitive mutant line, for human HeLa cells, and for sensitive CHO cells complemented by DNA fragments from the HeLa cells. (Adapted from Rubin JS, Joyner AL, Bernstein A, Whitmore GF: Molecular identification of a human DNA repair gene following DNA-mediated gene transfer. Nature 306:206-208, 1983, with permission.)

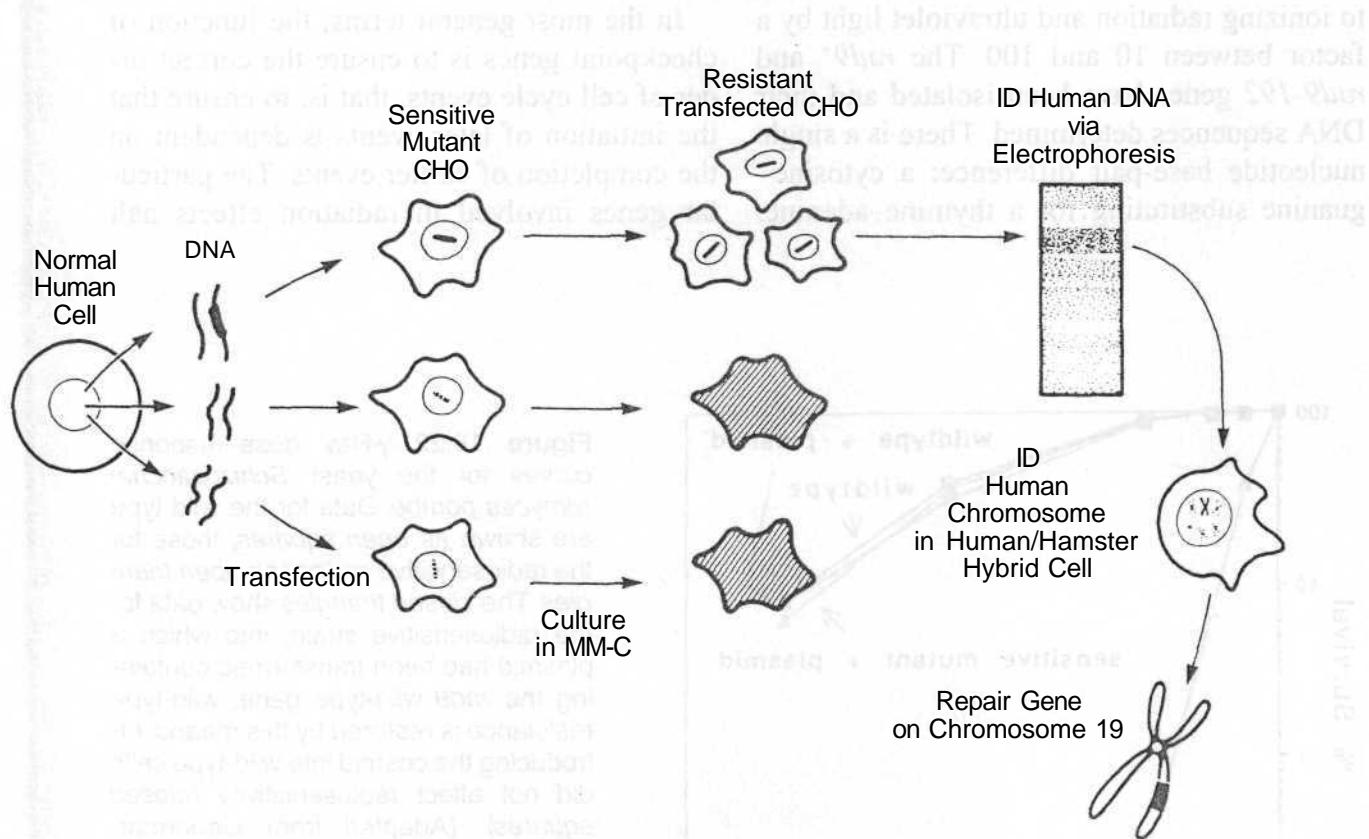
DNA from a human cell line (HeLa) was isolated and was transfected by the calcium phosphate precipitation method into the sensitive CHO cells. Cells were grown for several cycles in mitomycin C to select for repair proficient derivatives, and clones were chosen that had a sensitivity to mitomycin C close to that of the wild-type cells (Fig. 16.21). To demonstrate the successful integration of human DNA, use was made of the fact that the human genome contains a family of highly repetitive *Alu* sequences, which, under stringent hybridization conditions, can be used to establish the presence of human DNA within a CHO background. A Southern blot was used to determine the presence of human sequences. Independently derived transformants contained a common set of human-specific DNA restriction fragments, apparently associated with the DNA repair gene. The steps used in these experiments are illustrated in

Figure 16.21. The gene was subsequently cloned from resistant derivatives by a different group.

Techniques used included DNA-mediated gene transfer using the calcium phosphate precipitation technique, restriction fragment length analysis, and Southern blotting.

### The First Ionizing Radiation Repair Gene Isolated and Sequenced in Mammalian Cells (Thompson *et al*, 1990)

This was the first repair gene isolated using ionizing radiation to select for functionally complemented radiosensitive mutant CHO cells. In fact, the radiosensitivity to ionizing radiation of this cell line differs from the wild type by a factor of only 1.8. Much more radiosensitive mutants are known, but, in general, very radiosensitive lines are also defective in double-strand break repair, and it has



**Figure 16.21.** Illustrating the series of experiments in which DNA-mediated gene transfer was used to complement the repair-deficient CHO cells that were sensitive to mitomycin C. The repair gene from human cells was mapped to human chromosome 19. (From Rubin JS, Joyner AL, Bernstein A, Whitmore GF: Molecular identification of a human DNA repair gene following DNA-mediated gene transfer. *Nature* 306:206-208, 1983, with permission.)

proven difficult to transfect human DNA stably into these cells, perhaps for this reason.

A human fibroblast cDNA library in a cosmic vector was used to correct the radiation defect. The complementing human gene was cloned from the cosmic library of a transformant. The gene was given the designation *XRC-CCI* (x-ray repair cross complementing) and was assigned to the long arm of human chromosome 19.

Techniques included cDNA library, gene transfer, sequencing gel, Northern blotting, and gene mapping.

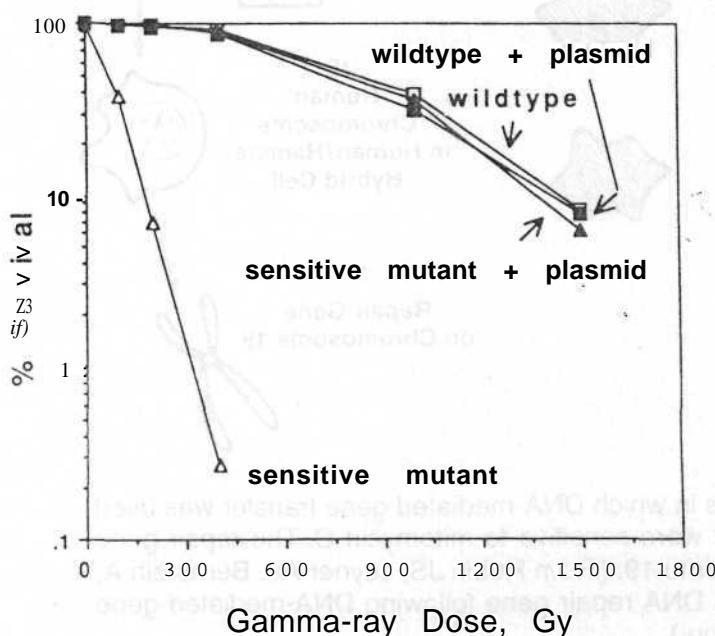
#### Molecular Checkpoint Genes Identified and Sequenced in Yeast (Murray *et al.*, 1991; Lieberman *et al.*, 1992)

Figure 16.22 shows ionizing radiation survival curves for the wild-type yeast *S. pombe* and for cells containing the mutant allele *rad9-192*. The mutant is more sensitive both to ionizing radiation and ultraviolet light by a factor between 10 and 100. The *rad9*<sup>w</sup> and *rad9-192* genes have been isolated and their DNA sequences determined. There is a single nucleotide base-pair difference: a cytosine-guanine substituting for a thymine-adenine.

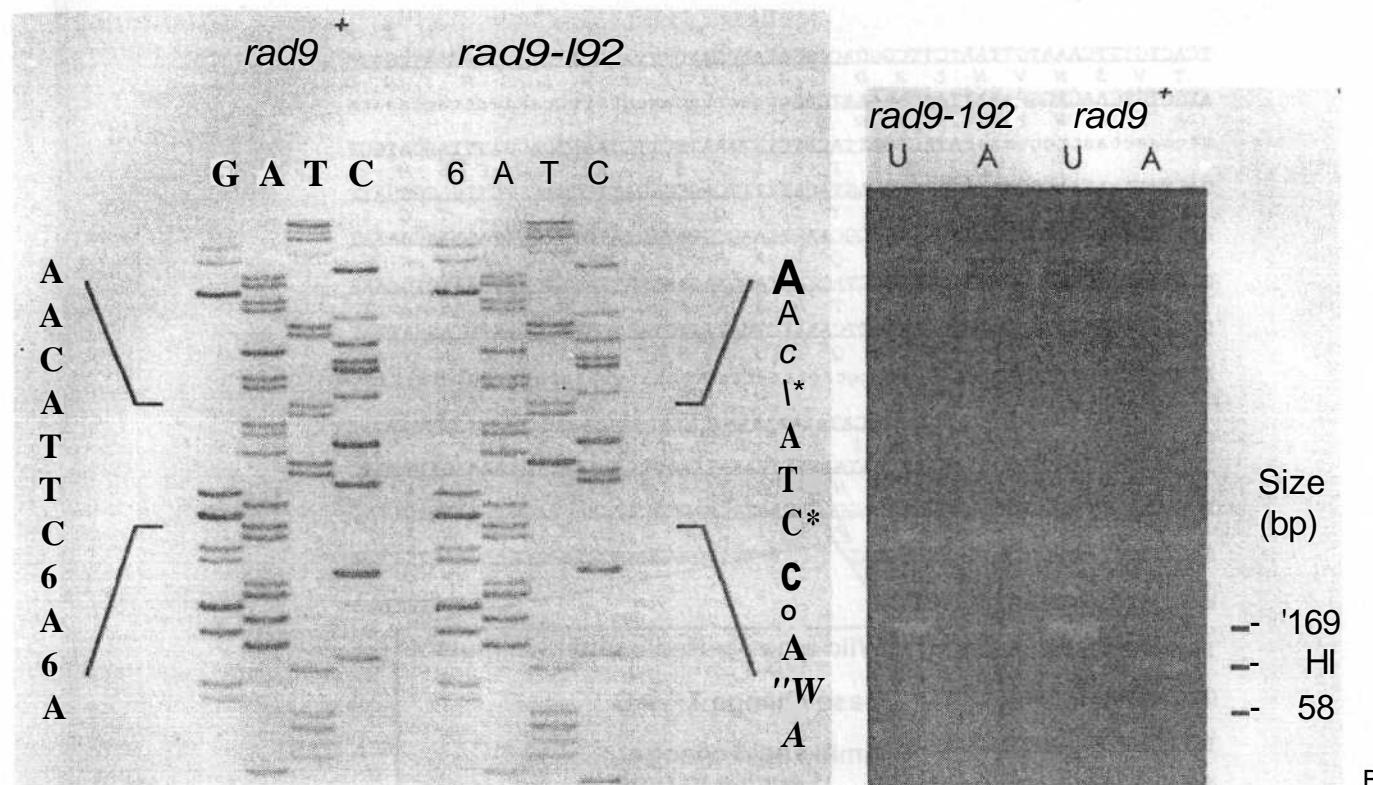
This would cause the coding for proline in place of leucine in the mutant *rac9*, which probably results in a dramatic change in the three-dimensional structure of the protein. This change evidently destroys protein activity and results in the dramatic increase in radiosensitivity exhibited by *rad9-192* cells. The alteration of 1 bp in a total of about 1,200 bp in the gene leads to this dramatic difference in radiosensitivity. Analysis of PCR-amplified genomic DNA including the mutated region in *rad9-192* cells, and the corresponding region in wild-type cells, confirms this difference. The sequencing gel is shown in Figure 16.23, and its interpretation is shown in Figure 16.24.

At first it was thought that the *rad9* gene was involved directly in DNA repair, and that the mutant cells were radiosensitive only because they were repair deficient. This is not the case. The *rad9* is a "molecular checkpoint gene."

In the most general terms, the function of checkpoint genes is to ensure the correct order of cell cycle events, that is, to ensure that the initiation of later events is dependent on the completion of earlier events. The particular genes involved in radiation effects halt



**Figure 16.22.**  $\gamma$ -Ray dose-response curves for the yeast *Schizosaccharomyces pombe*. Data for the wild type are shown as open squares, those for the radiosensitive mutant as open triangles. The closed triangles show data for the radiosensitive strain, into which a plasmid had been transformed containing the *rad9* wild-type gene; wild-type resistance is restored by this means, introducing the cosmid into wild-type cells did not affect radiosensitivity (closed squares). (Adapted from Lieberman HB, Hopkins KM, Laverty M, Chu HM: Molecular cloning and analysis of *Schizosaccharomyces pombe rad9*, a gene involved in DNA repair and mutagenesis. *Mol Gen Genet* 232:367-376, 1992, with permission.)



**Figure 16.23. A:** Sequencing gel. Part of a DNA sequencing gel indicating the single base-pair difference between the *Schizosaccharomyces pombe* *rad9*<sup>+</sup> and *rad9-192* genes. **B:** Polymerase chain reaction experiment. The single base-pair alteration within *rad9-192* falls within an *Au*l restriction enzyme cutting site normally found in wild-type genomic DNA. To confirm the sequence, polymerase chain reaction was used to amplify the region directly from wild-type and *rad9-192* mutant cell genomic DNAs. Resulting fragments were treated with *Au*l (A) or untreated (U) and then run through and visualized in an agarose gel. As can be seen, mutant genomic DNA is missing at the *Au*l site, confirming the existence of the mutation within the *rad9-192*-containing cells. (Courtesy of Drs. H. B. Lieberman and K. M. Hopkins)

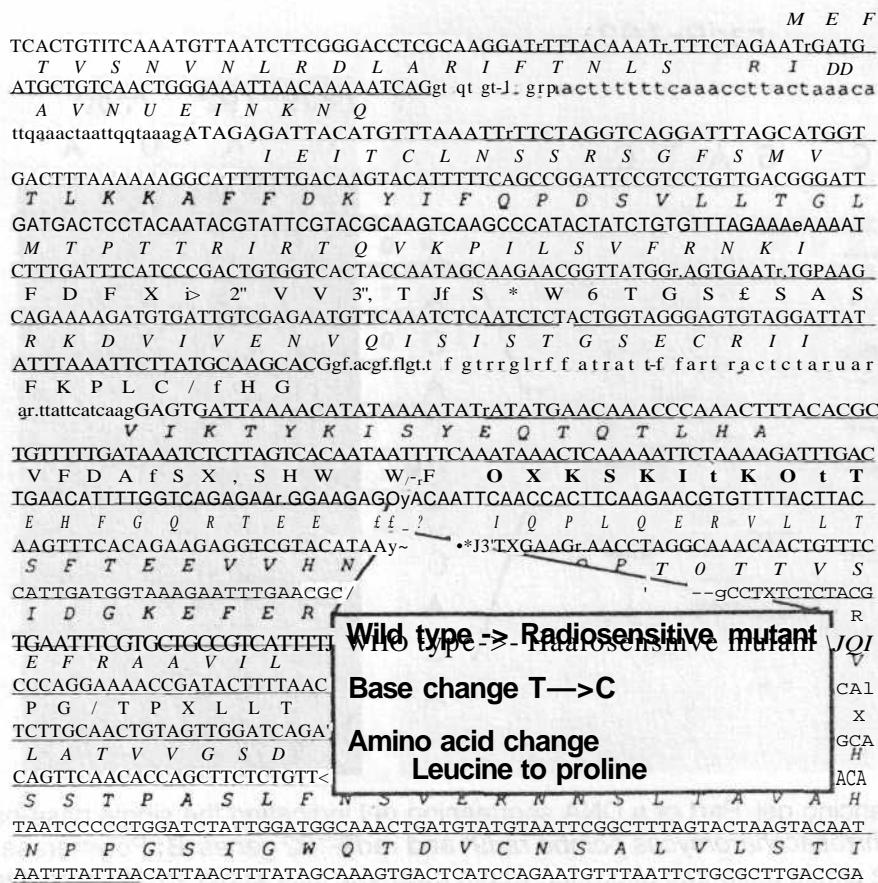
cells in the G2 phase, so that an inventory of chromosome damage can be taken and repair initiated and completed, before the complex task of mitosis is attempted. Mutant cells that lose this gene function move directly into mitosis with damaged chromosomes and are therefore at a higher risk of dying—hence their greater sensitivity to radiation, or for that matter to many other DNA-damaging agents. The first checkpoint control gene of this kind was identified in the yeast *S. cerevisiae*, by Weinert and Hartwell (1988), and coincidentally was also named *RAD9*.

Techniques used included a genomic DNA library, transformation, gene cloning, DNA sequencing, PCR, and sequencing gel.

### p53 as a Checkpoint Determinant in Mammalian Cells (Kuerbitz et al., 1992)

Cell-cycle checkpoints appear to be an important factor in determining radiosensitivity, that is, the fraction of cells surviving a given dose of radiation. Although much is known about checkpoint genes in yeast, relatively little is known about similar genes in mammalian cells.

It was found that cells lacking endogenous p53 gene function did not arrest in G1 after irradiation, but that transfection of wild-type p53 genes restored this property of arresting in G1. It was further hypothesized that the participation of p53 in this pathway suggests



**Figure 16.24.** The radioresistant wild-type *Schizosaccharomyces pombe* contains the *rad9<sup>+</sup>* gene. The radiosensitive strain contains the mutant allele *rad9-192*. Both genes contain an open-reading frame of 1,092 base pairs. A comparison of the DNA sequence of the two genes reveals a single nucleotide base-pair difference, a cytosine-guanine substituting for a thymine-adenine. This would cause the coding for proline instead of leucine in the mutant gene, promoting a dramatic change in the three-dimensional structure of the protein. (From Lieberman HB, Hopkins KM, Laverty M, Chu HM: Molecular cloning and analysis of *Schizosaccharomyces pombe* *rad9*, a gene involved in DNA repair and mutagenesis. Mol Genet 232:367-376, 1992, with permission.)

a mechanism for the contribution of p53 in tumorigenesis and genetic instability. Additional studies, however, are needed to determine whether p53 participates directly in checkpoint control and also to determine what its precise role might be.

Techniques included transfection of genes and Northern blotting.

#### Altered *ras* Oncogenes in Radiation-induced Tumors (Guerrero *et al.*, 1984)

Several studies have attempted to identify activated oncogenes in radiation-transformed

cells by various indirect methods. In the first paper from this group on this topic, the authors described the isolation of DNA from thymic lymphoma induced by  $\gamma$ -radiation in mice and were able to show that *K-ras* and *N-ras* were activated in 9 of 24 tumors. Of particular note was that seven of the tumors involved the same mutation. In a later publication, they showed that if neutrons were used, 4 of 25 tumors contained activated ras, but each of the mutations was different from the others and from those seen in the tumors induced by  $\gamma$ -rays. Because the activated oncogene could be identified in only a minority of tumors, it was concluded

that, although this altered gene may be involved in tumor development and progression, it is unlikely to be the sole causative agent.

Techniques used included PCR and Southern blotting.

### Characteristic Mutations in p53 in Lung Tumors from Uranium Miners (Vahakangas et al, 1992)

The identification of a radiation "signature" has been much discussed. It would clearly be of great importance to be able to say with certainty whether a given biologic lesion were produced by ionizing radiation as opposed to a chemical, for example, particularly if it was also possible to distinguish between high and low linear energy transfer radiations. "Molecular forensics" still may be a dream, but this paper indicates that one day it may be realized.

The study involved 19 uranium miners who developed lung cancer after prolonged exposure to high levels of radon. Mutations were not found in *Ki-ras*, but nine p53 mutations, including two deletions, were found in seven miners by direct DNA sequencing after PCR amplification of the tumor DNA. None of the mutations were guanine—cytosine to thymine—adenine transversions in the coding strand of the p53 gene, which is the most frequent base substitution associated with tobacco smoking. The pattern of changes seen in this suppressor gene may reflect the specific products of a high linear energy transfer  $\alpha$ -particle and is distinguishable from the other principal cause of lung cancer, namely tobacco smoke.

Techniques included PCR and sequencing gel.

### Parental Bias Indicating Genetic Susceptibility to the Induction of Lung Cancer (You et al, 1992)

The genetic basis of individual susceptibility to induced cancer was demonstrated dramatically in a recent paper involving the induction of cancer in mouse hybrids. A mouse strain with a low lung tumor suscep-

tibility (C3H) was crossed with a strain characterized by a high lung tumor susceptibility. Lung tumors, both spontaneous and chemically induced, were studied in the first-generation hybrids. The appearance of tumors was associated with mutations at codon 12 or codon 61 in the **Ki-ras** oncogene, leading to its activation. In 68 of 70 tumors studied, the mutation occurred in the allele from the susceptible parent. It was possible to identify the parental origin of mutated *ras* because of a characteristic 37-bp deletion in the second intron of the Ki-mms allele. This was a fortuitous occurrence that made this study possible.

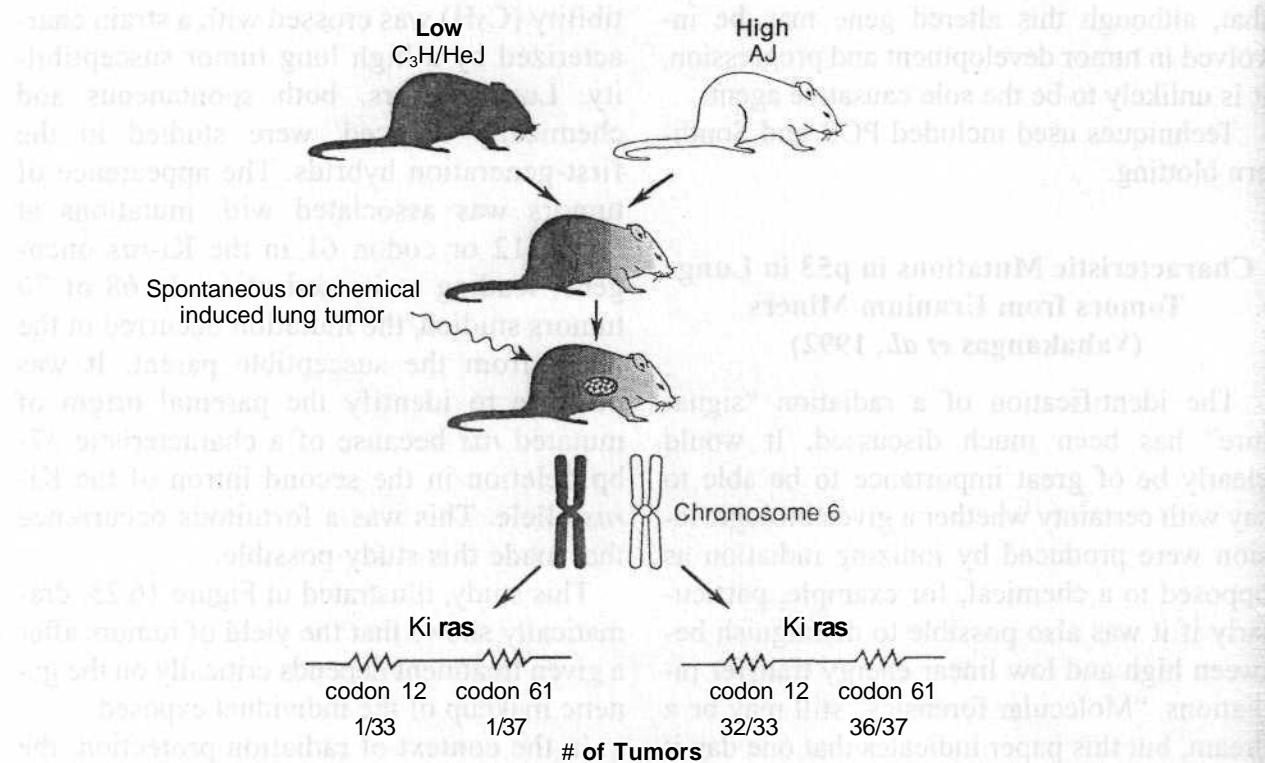
This study, illustrated in Figure 16.25, dramatically shows that the yield of tumors after a given treatment depends critically on the genetic makeup of the individual exposed.

In the context of radiation protection, the identification of susceptibility genes may allow the classification of both high- and low-risk groups. This may lead to questions of different exposure standards for different persons. At the present time only two special groups are recognized. It is recommended that exposure of the embryo or fetus is controlled more stringently because of the susceptibility to radiation damage, and female astronauts are limited to lower doses than males because of an increased cancer risk associated with a longer life expectancy and radiogenic sites such as the breast. Important and far-reaching ethical considerations would be involved if laboratory tests could be devised to identify susceptible persons, such as those heterozygotic for ataxia telangiectasia.

### Analysis of Radiation-induced Mutations (Thacker, 1986; Thacker et al, 1990)

One of the earliest applications of molecular techniques in radiobiology was the use of Southern blotting to analyze mutation spectra in somatic cells cultured *in vitro*.

The DNA from wild-type and mutant cells is digested with several different restriction enzymes and analyzed by the Southern blot



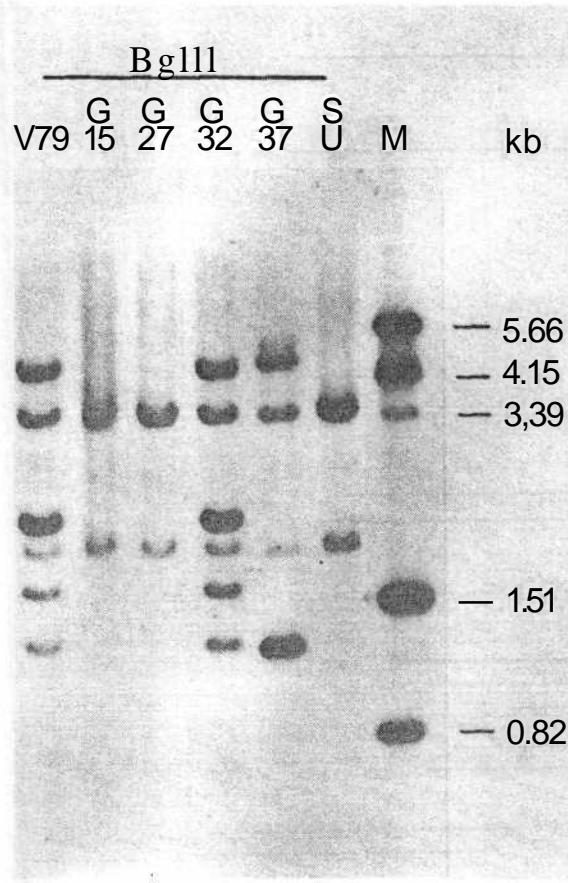
**Figure 16.25.** Illustration of an experiment that demonstrates the genetic basis of susceptibility to cancer. Two strains of mice were mated, one with a high and one with a low susceptibility to lung cancer. The F1 hybrids were examined for both spontaneous and chemically induced lung cancers. The tumors were associated with the activation of the *Ki-ras* oncogene, by a mutation in either codon 12 or codon 61. In 68 of 70 tumors the mutation was in the allele from the susceptible parent. (From You M, Wang Y, Stoner G, et al.: Parental bias of *Ki-ras* oncogenes detected in lung tumors from mouse hybrids. Proc Natl Acad Sci USA 89:5804-5808, 1992, with permission.) •

technique. Figure 16.26 shows such a blot after cutting DNA with *Bgl*/*II*. There are seven lanes: one for wild-type cells (V79), four for  $\gamma$ -ray-induced mutants (G), one for a spontaneous mutant(S), and a series of markers of known molecular weight(M).

DNA is electrophoresed in a gel, hybridized with the hamster HGPRT gene probe, and then autoradiographed.

In this *Bgl*/*II* digest, DNA from the wild-type cells containing an intact HGPRT gene shows up in six bands on the autoradiograph. Mutant G32 shows the same bands; that is, it is indistinguishable from the wild type, indicating that the mutation involves only a small change, possibly a point mutation. Changes of about 30 bp or less are not detectable by this technique. Each of the other  $\gamma$ -ray mutants, G15, G27, and G37, has

lost several bands, indicating the deletion of a significant portion of the gene. The HGPRT gene has been sequenced, and the position at which each restriction enzyme cuts has been mapped. With this information available, it is possible from a Southern blot analysis to show the size and position of partial deletions and rearrangements of the HGPRT gene in a series of mutants produced by  $\gamma$ -rays and  $\alpha$ -particles. An example from a 1990 paper by Thacker and his coworkers is shown in Figure 16.27. Some radiation-induced mutations involve a change too small to be detected by Southern blotting, but most involve a sizable deletion of part or all of the gene. Figure 16.27 suggests that the deletions produced by  $\alpha$ -particles are larger (on average) than those produced by  $\gamma$ -rays.



**Figure 16.26.** Hybridization analysis (Southern blot) of genomic DNA from V79 parent cells, four mutants induced by  $\gamma$ -rays, and one spontaneous mutant digested with *Bgl*/II. Pseudogene fragments are at 3.4 and 1.9 kilobases (*Bgl*/II). *M*, molecular weight markers (From Thacker J: The nature of mutants induced by ionizing radiation in cultured hamster cells. *Mutat Res* 160: 267-275, 1986, with permission.)

The technique used in this study was Southern blotting.

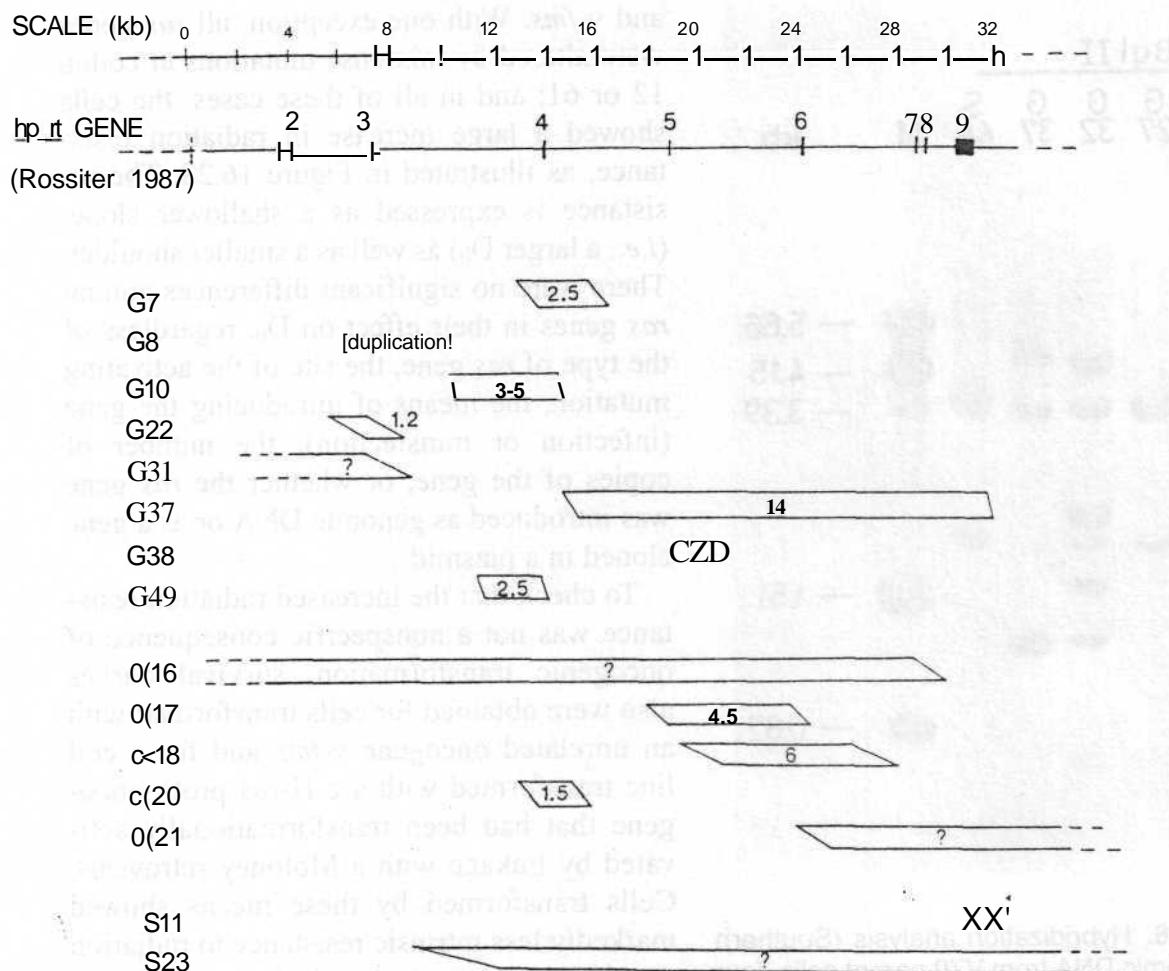
#### Oncogenes and Radioresistance (Sklar, 1988; McKenna *et al.*, 1990, 1991)

The first paper to show that transfecting an activating oncogene into cells could result in enhanced radioresistance was published by Sklar in 1988. NIH 3T3 cells were transfected by the calcium phosphate precipitation technique with either genomic DNA containing human *c-H-ras* from bladder cancer or *N-ras* from Hodgkin's disease or HL 60 leukemia or cloned oncogenes including *c-R-ras*, *v-H-ras*,

and *v-fms*. With one exception, all *ras* genes were altered by missense mutations at codon 12 or 61; and in all of these cases, the cells showed a large increase in radiation resistance, as illustrated in Figure 16.28. The resistance is expressed as a shallower slope, (*i.e.*, a larger *D<sub>0</sub>*) as well as a smaller shoulder. There were no significant differences among *ras* genes in their effect on *D<sub>0</sub>*, regardless of the type of *ras* gene, the site of the activating mutation, the means of introducing the gene (infection or transfection), the number of copies of the gene, or whether the *ras* gene was introduced as genomic DNA or as a gene cloned in a plasmid.

To check that the increased radiation resistance was not a nonspecific consequence of oncogenic transformation, survival curves also were obtained for cells transformed with an unrelated oncogene *v-fms* and for a cell line transformed with a *c-H-ras* proto-oncogene that had been transformationally activated by linkage with a Moloney retrovirus. Cells transformed by these means showed markedly less intrinsic resistance to radiation than cells transformed with the activated *ras* genes.

In a later and more detailed paper, McKenna and his colleagues in 1990 investigated the effect of oncogenes on the radiosensitivity of primary rat embryo cells, which have the advantages of being diploid and being in culture for only a few cell generations. Cell lines were generated from primary rat embryo fibroblasts by DNA-mediated gene transfer of a plasmid bearing the *v-myc* or *c-E-ras* gene, using calcium phosphate precipitation. It was found that the activated *H-ras* oncogene was associated with radiation resistance after transformation, but that the effect of the oncogene itself was small and consisted of a change of slope of the radiation survival curve at high doses but little or no change at lower doses in the shoulder region of the curve. By contrast, cells transformed by *H-ras* plus *v-myc* were characterized by increased resistance to low doses in the shoulder region of the radiation survival curve as well as a shallower slope at



**Figure 16.27.** Size and position of partial deletions/rearrangements of the hamster hprt gene in a series of mutants produced by  $\gamma$ -rays (G) or by  $\alpha$ -particles or that arose spontaneously (S). A scale with arbitrary positioning is shown at the top, and the next line shows the approximate locations of the known exons of the gene. The mutants analyzed are listed below with the approximate sizes, in thousands of base pairs, of deletions {open boxes} shown if possible; the angles of the vertical lines indicating breakpoints are drawn to include the uncertainties of location. Dashed lines indicate that the deletion extends into flanking DNA. (From Thacker J, Fleck EW, Morris T, Rossites BJF, Morgan TL: Localization of deletion breakpoints in radiation-induced mutants of the hprt gene in hamster cells. *Mutat Res* 232:163-170, 1990, with permission.)

high doses. The  $\lambda$ -*myc* oncogene by itself had essentially no effect on radiation survival. This change of inherent radioresistance in the shoulder region is more relevant to the dose range of importance in clinical radiation oncology.

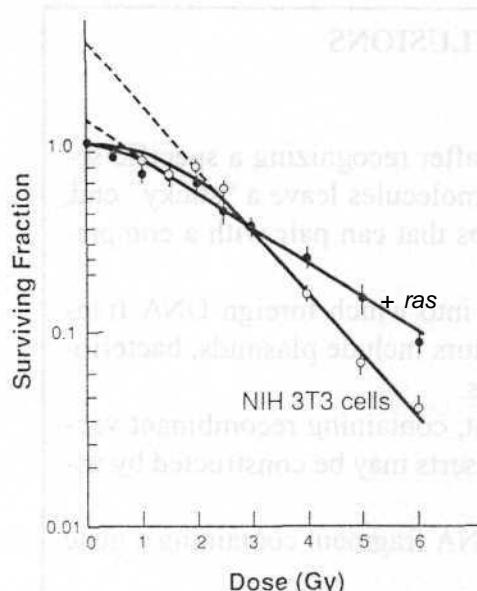
The mechanism for the increased radioresistance conferred by oncogenes is not entirely clear. McKenna and his colleagues have suggested, however, that cells cotransfected with *ms* and *myc* are characterized by a significantly longer G<sub>2</sub> phase arrest after irradiation,

and that this may be the basis of their increased radioresistance.

Techniques used include DNA-mediated gene transfer, genomic library construction, and plasmids as vectors.

#### Cloning and Sequencing the Gene for Ataxia Telangiectasia (Savitsky et al., 1995)

Ataxia telangiectasia is inherited in a monogenic, autosomal recessive manner. It is



**Figure 16.28.** Radiation survival curves for NIH 3T3 cells (open symbols) and similar cells transformed by missense mutation-activated *ras* gene (closed symbols). The increase in intrinsic radiation resistance was the same regardless of the source of the *ras* gene, the copy number, or the means of introducing the gene. (Adapted from Sklar MD: The *ras* oncogenes increase the intrinsic resistance of NIH 3T3 cells to ionizing radiation. Science 239:645-647, 1988, with permission.)

a disorder characterized by progressive neurologic degeneration, growth retardation, premature aging, telangiectasia, specific immunodeficiencies, high sensitivity to ionizing radiation, gonadal atrophy, genomic instability, checkpoint control deficiencies, and defective telomere metabolism.

The gene responsible for the disease ataxia telangiectasia was identified by positional cloning. The major difficulty in cloning the gene was that the disease did not show any link with a germ line chromosomal rearrangement, which could have pinpointed the position of a gene responsible. Several approaches were tried in an effort to clone the gene. First, a functional approach based on complementation of the cellular phenotype of radiosensitivity by gene transfer failed. A genetic linkage analysis approach localized the gene on chromosome 11 q22-23 locus. The size of the region was 6 to 7 cM (centiM-

organ), too large to consider searching for candidate gene sequences.

Several groups made efforts to increase marker density within the chromosomal segment defined by the closest flanking markers in order to obtain a more accurate gene localization. Using the increased number of markers, the location of the ataxia telangiectasia locus was narrowed to a region of about 3 Mb of DNA. The positional cloning approach was finally applied to identify the gene. Long-range cloning of the locus was performed by constructing a contig of yeast artificial chromosome clones across the interval and flanking sequences. A high-density marker map was developed, and the locus was confined to a region of 500 kb. Further localization of the gene was accomplished by hybridization of the cDNA, which was confined to a region of 150 kb, spanning the ATM gene. The cDNA of the locus in patients with ataxia telangiectasia showed aberrant message size and different mutations. The transcript is 13 kb with an open reading frame of 9.5 kb.

This gene is of considerable interest in radiation biology. The ATM protein contributes to the induction of c-Abl activity, a tyrosine kinase activated by ionizing radiations. The ATM protein itself is activated by ionizing radiation, and phosphorylation of p53 by ATM in response to DNA damage has been established. The gene shows homology to TEL1 (a gene in yeast coding for telomerase), and inactivation of ATM results in defective telomere metabolism, a possible link for cellular transformation and malignancy. Individuals homozygous for ATM have serious clinical problems and are extremely radiosensitive and cancer prone. Of wider interest are those who are heterozygotic for ataxia telangiectasia, who comprise 1 to 3% of the U.S. population. Epidemiologic studies suggest that they are more prone to develop cancer, especially breast cancer. It has been suggested that they may be more susceptible to radiation-induced cancer, too, but this remains to be proven.

The technique used was positional cloning.

## SUMMARY OF PERTINENT CONCLUSIONS

### Terms and Techniques

- Restriction endonucleases are enzymes that cleave DNA after recognizing a specific sequence. Those most useful for constructing recombinant molecules leave a "sticky" end, that is, a single-strand overhang of two to four nucleotides that can pair with a complementary strand.
- A **vector** is an autonomously replicating DNA molecule into which foreign DNA fragments are inserted and then propagated in a host cell. Vectors include plasmids, bacteriophage, cosmids, yeast artificial chromosomes, and viruses.
- A **library** is a collection of cells, usually bacteria or yeast, containing recombinant vectors, carrying DNA inserts from a different species. The inserts may be constructed by using restriction enzyme digested genomic DNA or cDNA.
- A **host** is used to grow (i.e., to multiply) or to express a DNA fragment containing a gene of interest.
- In **agarose gel electrophoresis**, DNA or RNA molecules can be separated according to size by causing them to move through a matrix composed of purified agar under the influence of an electric field.
- **Polymerase chain reaction** (PCR) is a procedure that enzymatically amplifies the number of copies of a DNA sequence, up to several thousand base pairs, through repeated replication by DNA polymerase. (A DNA polymerase is an enzyme that catalyzes the addition of nucleotides to a growing DNA molecule.)
- Genes may be cloned by **functional complementation** (i.e., putting a functional version of a defective gene into cells to correct the phenotype associated with the defect) by **hybridization** or by making an **antibody probe** or **oligonucleotide probe**.
- **Positional cloning** is a strategy used to clone a gene for which no information is available about its protein product. This is true of most human inherited diseases, and so this strategy has been used to clone, for example, many of the tumor suppressor genes.
- Genes may be "mapped," that is, their position in the genome identified by **in situ hybridization**, by **Southern blotting**, by **chromosome walking** if a flanking sequence is identified, and by **contiguous mapping** if sequence data from large adjacent regions of the genome are aligned.
- Once a gene is isolated, it can be **sequenced** by one of several methods, the most common of which is the **chain-termination method**.
- The amino acid sequences within a protein can be determined from the corresponding DNA sequence using the known genetic triplet code.
- A newly acquired sequence can be compared with the international database to seek **homologies** with known genes or proteins.
- Structural variations in DNA caused by point mutations, deletions, or insertions can result in restriction fragments of different lengths, which can often be detected by Southern blotting. These are known as **restriction fragment length polymorphisms**, which can be used as genetic markers to map genes to specific chromosomal locations and identify aberrant genes causing disease.
- A mutation involving only a single base-pair difference between two short **single-stranded** DNA molecules can be detected by the technique of **single-stranded conformation polymorphism**.
- In studying the level at which a promoter is working, it is often useful to use a simple bacterial assay (such as the CAT assay) instead of making a quantitative assessment of the protein produced by the gene.

### Projects Of Importance to Radiobiology Employing Molecular Techniques

- The first **human repair gene** isolated was involved in repairing damage induced by mitomycin C. The techniques of DNA-mediated gene transfer and functional complementation were used; that is, the gene from a human cell (HeLa) was used to correct the repair deficiency in a Chinese hamster cell.
- The first **human ionizing radiation repair gene** was isolated, characterized, and sequenced by using a human cDNA library in a cosmic vector to correct the radiation repair deficiency in Chinese hamster cells. The gene was assigned to the long arm of human chromosome 19.
- A number of **molecular checkpoint genes** have been identified and sequenced in yeast. These genes function by arresting cells in G2 phase after irradiation to allow repair of damaged DNA before cells enter mitosis; cells in which these genes are not functional exhibit very much more radiosensitivity.
- Human cells that lack endogenous p53 genes did not arrest in Gi phase after irradiation transfection of wild-type p53 genes restored this property. It appears that one of the functions of **p53** may be a **checkpoint regulator**.
- **K-ras** and **N-ras** were **activated** in **9 of 24 thymic lymphomas** induced by  $\gamma$ -rays, and 4 of 25 tumors induced by neutrons. Because the activated oncogenes are present in only a minority of tumors, it is likely to be a late step in tumor progression rather than a causative event.
- By direct sequencing of the p53 tumor-suppressor gene in lung tumors from uranium miners, a characteristic spectrum of mutations was observed that was quite different from that produced by tobacco smoke. This may reflect the specific products of high linear energy transfer  $\alpha$ -particles, that is, a **radiation "signature."**
- **Genetic susceptibility** to lung cancer, spontaneous or induced, was shown by cross-breeding two mouse strains, one with high and one with low susceptibility to lung cancer, and showing that the mutation in the **KA-ras** oncogene associated with the tumors almost always occurred in the allele from the susceptible parent.
- **Southern blot analysis** has been used to show that most mutations produced by ionizing radiation (at least in somatic cells cultured *in vitro*) are large deletions. There is some suggestion that the spectrum of mutations is different for  $\alpha$ -particles than for  $\gamma$ -rays, with a preponderance of larger deletions for the densely ionizing radiation.
- By transferring cloned genes or genomic DNA from tumor cells, it was shown that **activated oncogenes** could **confer resistance** to cell killing by radiation. The combination of **ras** plus **myc** was more effective than either alone.
- The technique of **positional cloning** was used to clone and sequence the gene for ataxia telangiectasia. It is a large gene, 66 exons and 150 kb. This gene influences radiation sensitivity and cancer susceptibility

### GLOSSARY OF TERMS

**alleles** Alternate forms of a gene or DNA sequence on the two homologous chromosomes of a pair.

**amino acids** The 20 basic building blocks of proteins.

**ampicillin** An antibiotic that prevents bacterial growth.

**amplify** To increase the number of copies of a DNA sequence by inserting into a cell *in vivo* or *in vitro* by the polymerase chain reaction.

**anneal** The pairing of complementary DNA or RNA sequences, *via* hydrogen bonding, to form a double-stranded polynucleotide.

**antibiotic** Compounds that inhibit the growth of or kill microorganisms.

**antibiotic resistance** The ability of a microorganism to disable an antibiotic or prevent transport of the antibiotic into the cell.

**antibody** An immunoglobulin protein produced by B lymphocytes that binds to a specific antigen.

**monoclonal antibodies** Immunoglobulin molecules of single-epitope specificity.

**polyclonal antibodies** A mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different epitope.

**antigen** Any foreign substance that elicits an immune response by stimulating the production of antibodies.

**bacteriophage** A virus that infects bacteria. Altered forms are used as vectors for cloning DNA.

**bacterium** A single-cell prokaryotic organism.

**base pair** A pair of complementary nitrogenous bases in a DNA molecule: adenine-thymine or guanine-cytosine. It is also a measure of the length of DNA.

**B lymphocyte** A white blood cell responsible for production of antibodies involved in the humoral immune response.

**cDNA** See **DNA**.

**cDNA library** See **library**.

**centromere** The central portion of the chromosome to which the spindle fibers attach during mitotic and meiotic division.

**codon** A group of three nucleotides that specifies the addition of one of the 20 amino acids during translation of mRNA into a polypeptide.

**initiation codon** The mRNA sequence AUG, which codes for methionine and which initiates translation.

**termination (stop) codon** Any of three mRNA sequences (UGA, UAG, UAA) that do not code for an amino acid and thus signal the end of protein synthesis.

**colony** A group of identical cells derived from a single ancestor cell.

**contig** A collection of DNA sequences or yeast artificial chromosomes or cosmids that overlap at portions of their ends.

**digest** To cut DNA molecules with one or more restriction endonucleases.

**DNA (deoxyribonucleic acid)** An organic acid composed of four nitrogenous bases (adenine, thymine, cytosine, and guanine) linked via sugar and phosphate units. DNA is the genetic material of most organisms and usually exists as a double-stranded molecule in which two antiparallel strands are held together by hydrogen bonds between adenine-thymine and cytosine-guanine base pairs.

**cDNA (copy DNA)** DNA synthesized from an RNA template using reverse transcriptase.

**DNA fingerprint** A unique pattern of DNA fragments identified by Southern hybridization or by polymerase chain reaction.

**DNA polymorphism** One or two or more alternate forms of a chromosomal locus that differ in nucleotide sequence or have variable numbers of repeated nucleotide units.

**DNA sequencing** Procedures for determining the nucleotide sequence of a DNA fragment.

**electrophoresis** The technique of separating charged molecules in a matrix to which an electrical field is applied.

**agarose gel electrophoresis** A matrix composed of purified agarose is used to separate larger DNA and RNA molecules ranging from 100 to 20,000 nucleotides.

**polyacrylamide gel electrophoresis** Electrophoresis through a matrix composed of synthetic polymer, used to separate small DNA, or RNA, molecules (up to 1,000 nucleotides) or proteins.

**pulse-field electrophoresis** The current is alternated between pairs of electrodes set at angles to one another to separate very large DNA molecules of up to 10 million nucleotides.

**electroporation** High-voltage pulses of electricity are used to open pores in the cell membrane through which foreign DNA can pass.

**Escherichia coli** A bacterium found in the human colon that is widely used as a host for molecular cloning experiments.

**ethidium bromide** A fluorescent dye used to stain DNA and RNA, which intercalates between nucleotides and fluoresces if exposed to ultraviolet light.

**exon** That portion of a gene expressed in mature mRNA.

**flanking region** The DNA sequences extending on either side of a specific locus or gene.

**gene** A locus on a chromosome that encodes a specific protein or several related proteins.

**dominant gene** A gene whose phenotype is expressed if it is present in a single copy.

**recessive gene** The phenotype expressed only if both copies of the gene are mutated or missing.

**gene amplification** The presence of multiple copies of a gene. This is one mechanism by which protooncogenes are activated to result in neoplasia.

**gene expression** The process of producing a protein from its DNA- and mRNA-coding sequences.

**genetic code** The three-letter code that translates a nucleic acid sequence into a protein sequence.

**genome** The genetic complement contained in the chromosomes of a given organism.

**hybrid** The offspring of two parents differing in at least one genetic characteristic.

**hybridization** The hydrogen bonding of complementary DNA or RNA sequences to form a duplex molecule.

**Northern blotting** A procedure in which RNA fragments are transferred from an agarose gel to a nitrocellulose filter, where the RNA is then hybridized to a radioactive probe.

**Southern blotting** A procedure in which DNA restriction fragments are transferred from an agarose gel to a nitrocellulose filter, where the denatured DNA is then hybridized to a radioactive probe.

**library** A collection of cells (usually bacteria or yeast) that have been transformed

with recombinant vectors carrying DNA inserts from a single species.

**cDNA library** A library composed of complementary copies of cellular mRNAs (*i.e.*, the exons without the introns).

**expression library** A library of cDNAs whose encoded proteins are expressed by specialized vectors.

**genomic library** A library composed of fragments of genomic DNA.

**ligase** An enzyme that catalyzes a reaction that links two DNA molecules by the formation of a phosphodiester bond.

**ligation** The process of joining two or more DNA fragments.

**microinjection** Introducing DNA into a cell using a fine microcapillary pipet.

**mitosis** Replication of a cell to form two identical daughter cells.

**mutation** An alteration in DNA structure or sequence of a gene.

**point mutation** A change in a single base pair in a gene.

**nucleotide** A building block of DNA and RNA.

**complementary nucleotides** Members of the pairs adenine-thymine, adenine-uracil, and guanine-cytosine that have the ability to hydrogen bond to one another.

**oncogene** A gene that contributes to cancer formation when mutated or inappropriately expressed.

**cellular oncogene (protooncogene)** A normal gene that if mutated or improperly expressed contributes to the development of cancer.

**myc** A nuclear oncogene involved in immortalizing cells.

**ras** An oncogene that can induce the malignant phenotype; it converts guanosine triphosphate to guanosine diphosphate, a step in signal transduction.

**plaque** A clear spot on a lawn of bacteria or cultured cells where cells have been lysed by viral infection and replication.

**plasmid** A circular DNA molecule, capable of autonomous replication, which may typ-

ically carry one or more genes encoding antibiotic resistance.

**polymerase** An enzyme that catalyzes the addition of multiple subunits to a substrate molecule.

**DNA polymerase** Synthesizes a double-stranded DNA molecule using a primer and DNA as a template.

**RNA polymerase** Transcribes RNA from a DNA template.

**Taq polymerase** A heat-stable DNA polymerase used in polymerase **chain** reaction.

**polymerase chain reaction** A procedure that enzymatically amplifies a DNA sequence through repeated replication by DNA polymerase

**positional cloning** A strategy to clone a gene where the protein product is not known.

**primer** A short DNA or RNA fragment annealed to single-stranded DNA.

**probe** A single-stranded DNA (or RNA) that has been radioactively labeled and is used to identify complementary sequences.

**reading frame** A series of triplet codons beginning from a specific nucleotide.

**open reading frame** A long DNA sequence, uninterrupted by a stop codon, that encodes part or all of a protein.

**recombinant DNA** The process of cutting and recombining DNA fragments.

**restriction endonuclease (enzyme)** A class of endonucleases that cleaves DNA after recognizing a specific sequence.

**restriction fragment length polymorphism** Differences in nucleotide sequence between alleles that result in restriction fragments of varying lengths.

**retrovirus** A class of viruses whose genome consists of RNA and that utilizes the enzyme reverse transcriptase to copy its genome into a DNA intermediate, which integrates into the chromosome of a host cell.

**reverse transcriptase** An enzyme that synthesizes a complementary DNA strand from an RNA template.

**RNA (ribonucleic acid)** An organic acid composed of repeating nucleotide units of adenine, guanine, cytosine, and uracil,

whose ribose components are linked by phosphodiester bonds.

**messenger RNA (mRNA)** The class of RNA molecules that copies the genetic information from DNA, in the nucleus, and carries it to ribosomes, in the cytoplasm.

**transfer RNA (tRNA)** Small RNA molecules that transfer amino acids to the ribosomes during protein synthesis.

*Saccharomyces cerevisiae* Brewer's yeast.

**selectable marker** A gene whose expression makes it possible to identify cells that have been transformed or transfected with a vector containing the marker gene. It is usually a gene for resistance to an antibiotic.

**somatic cell** Any cell other than a germ cell that composes the body of an organism and that possesses a set of multiploid chromosomes.

**stem cell** An undifferentiated cell that gives rise to one or more types of specialized cells.

**sticky end** A single-stranded nucleotide sequence produced if a restriction endonuclease cleaves off center in its recognition sequence.

**stringency** Reaction conditions, such as temperature, salt, and pH, that dictate the annealing of single-stranded DNA/DNA, DNA/RNA, and RNA/RNA hybrids. At high stringency, duplexes form only between strands with perfect one-to-one complementarity: Lower stringency allows annealing between strands with less than a perfect match between bases.

**template** An RNA or single-stranded DNA molecule upon which a complementary nucleotide strand is synthesized.

**transcription** The process of creating a complementary RNA copy of DNA.

**transfection** The uptake and expression of foreign DNA by cultured eukaryotic cells.

**transformation** In higher eukaryotes, the conversion of cultured cells to a malignant phenotype. In prokaryotes, the natural or induced uptake and expression of a foreign DNA sequence.

**translation** The process of converting the genetic information of an mRNA on ribosomes into a polypeptide.

**vector** An autonomously replicating DNA molecule into which foreign DNA fragments are inserted and then propagated in a host cell.

**yeast artificial chromosome** A vector used to clone DNA fragments of up to 400,000 bp, which contains the minimum chromosomal sequences needed to replicate in yeast.

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## Cancer Biology

### MECHANISMS OF CARCINOGENESIS

#### ONCOGENES

#### TUMOR SUPPRESSOR GENES

#### THE PARADIGM OF RETINOBLASTOMA

#### SOMATIC HOMOZYGOSITY

#### MULTISTEP NATURE OF CANCER

#### FUNCTION OF ONCOGENES AND TUMOR

#### SUPPRESSOR GENES

#### GATEKEEPERS AND CARETAKERS

#### MISMATCH REPAIR

#### TELOMERES AND CANCER

### SIGNAL TRANSDUCTION

#### RADIATION-INDUCED SIGNAL

#### TRANSDUCTION

#### THE CELL CYCLE

#### CYCLINS AND KINASES

#### CHECKPOINT GENES

#### CANCER GENETICS

#### ATAXIA TELANGIECTASIA AND CANCER

#### GENOMIC IMPRINTING

#### TRANSGEMIC MICE

#### SUMMARY OF PERTINENT CONCLUSIONS

Cancer is thought to be a clonal disorder, because there is evidence that most cancers arise from a single cell that has suffered a disruption in its regulatory mechanisms for proliferation and self-elimination.

Malignant cells differ from their normal tissue counterparts in a number of ways:

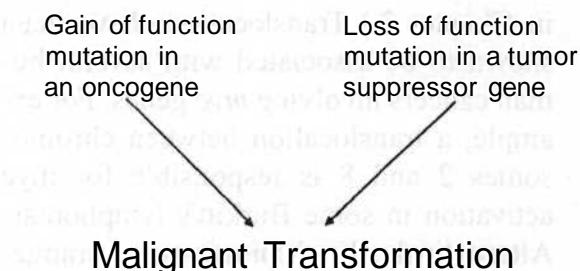
- They are often immortal, or at least have the ability to divide many more times than cells from normal somatic tissues, which tend to senesce after 50 or 60 doublings.
- They often grow more rapidly than cells of the same tissue of origin—though not necessarily faster than the normal cell can divide if given the stimulus.
- They fail to show normal cell-to-cell interactions, which results in their ability to invade, metastasize, and grow in a foreign environment. The change from a completely normal tissue to a frankly malignant metastasizing tumor frequently occurs in a number of discreet steps over a period of time. These "steps," which are the result of muta-

tions, deletions, or gene changes in growth regulators, may occur spontaneously as a consequence of random errors, or they may result from exposure to agents as diverse as chemical mutagens, ionizing radiations, ultraviolet light, and viruses.

### MECHANISMS OF CARCINOGENESIS

Carcinogenesis appears to be a multistep process with multiple genetic alterations occurring over an extended period of time; at least, that is how it appears. Sometimes these genetic alterations are carried in the germline, as for example in the cancer-predisposing syndromes such as retinoblastoma; however, heritable mutations are the exception. Most alterations that lead to cancer are acquired in the form of somatic mutations; chromosomal translocations, deletions, inversions, amplifications, or simply point mutations.

The control of cell proliferation is the consequence of signals affecting cell division

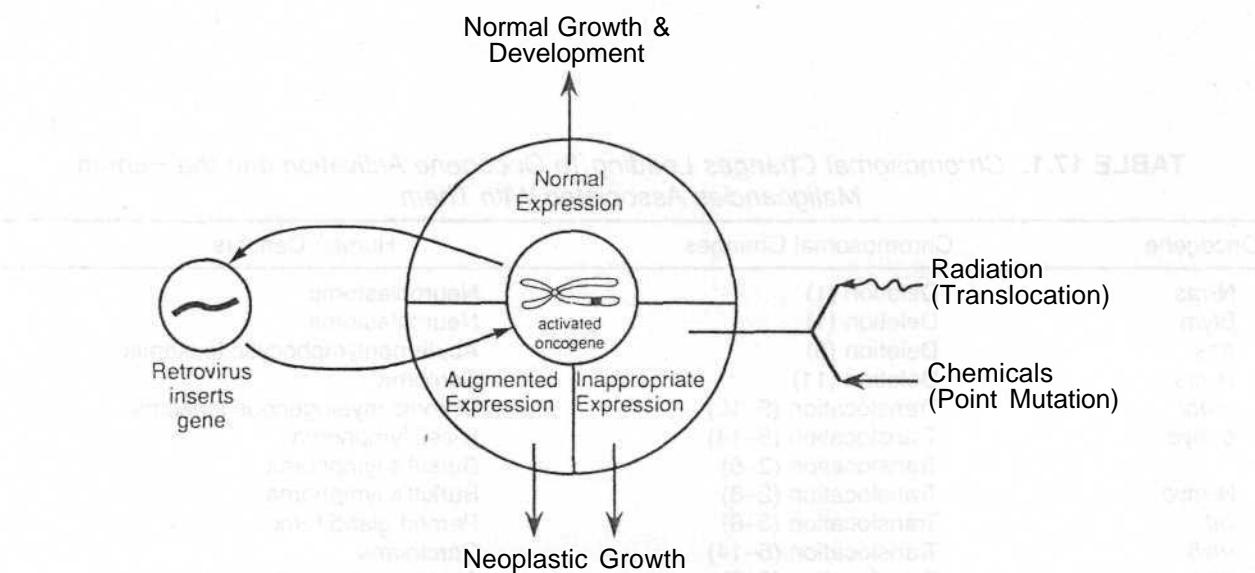


**Figure 17.1.** The process of malignant transformation may result from either a gain-of-function mutation that activates an oncogene, or a loss-of-function mutation in a tumor-suppressor gene.

and differentiation. These signals may be positive or negative, and the acquisition of tumorigenicity results from changes that affect these control points. The conversion of a cell to a malignant state may result from either gain-of-function mutations, which can activate oncogenes, positive effectors of transformation; or loss of function of a tumor-suppressor gene product, which is a negative growth regulator. This is illustrated in Figure 17.1.

ONCOGENES

Oncogenes were first discovered from a study of retroviruses (*i.e.*, viruses whose genomes are composed of RNA) that cause cancers in animals. The retroviruses contain modified cellular genes captured from the genomes of their vertebrate hosts. Cancer caused by a retrovirus, then, is mediated through cellular genes that have been mutated or changed. Normal versions of these genes (protooncogenes) are present in every mammalian cell, and many have been shown to function in regulating cell growth. Infection of cells by these viruses leads to integration of the viral oncogene into the host genome, in which it is expressed at high levels leading to overproliferation, the signature of cancer. Although a virus causes cancer by *inserting* an oncogene from its own genome into that of the cell, the mechanism of transformation by radiation or chemicals is to cause changes in a normal protooncogene *indigenous* to that cell, causing it to be activated. The notion of oncogenes helps explain why agents as diverse as radiation, chemicals, and viruses can all produce tumors that are indistinguishable one from another. This is illustrated in Figure 17.2.



**Figure 17.2.** The way in which the concept of oncogenes provides a ready answer for how agents as diverse as viruses, radiations, and chemicals all can induce tumors that are essentially indistinguishable from another. The retrovirus inserts a gene; a chemical may activate an endogenous oncogene by a point mutation; radiation may do the same by, for example a translocation. (Adapted from Bishop JM: Cellular oncogene retroviruses. Ann Rev Biochem 52:301-354, 1983, with permission.)

There are three principal mechanisms by which protooncogenes can be activated to produce a malignant cell:

1. A point mutation can occur, changing a single base pair, which subsequently produces a protein with a single amino acid change. For example, a point mutation in *k-ras* is found in the cancer cells of most patients suffering from colon cancer.
2. A chromosomal rearrangement or translocation can occur, often placing a protooncogene next to a strong promotor sequence, leading to its overexpression, or producing a new fusion gene whose product acquires a new transforming activity. Ionizing radiations are highly effective at producing DNA breaks. These chromosomal breaks may rejoin in such a way as to form a dicentric chromosome, which is almost always lethal to the cell. An approximately equal number of symmetric chromosome translocations may occur, which are not lethal and are much more difficult to see, except with the new techniques of chromosome painting. (Dicentrics and translocations are explained
3. Gene amplification, in which many new extra copies of a protooncogene are generated in a cell, is associated with the activation of oncogenes in several cancers. The presence of multiple copies of a protooncogene leads to its overexpression. Gene amplification of N-myc is characteristic of many neuroblastomas.

in Chapter 2.) Translocations have been shown to be associated with several human cancers involving *myc* genes. For example, a translocation between chromosomes 2 and 8 is responsible for myc activation in some Burkitt's lymphomas. Alternatively, the chromosome rearrangement may result in a partial deletion, as in the activation *ofc-fos*.

Table 17.1 is a summary of some known oncogenes and the human cancers associated with them, as well as the characteristic chromosomal changes seen.

Today, close to 100 oncogenes have been identified in human cancers and are at various stages of characterization, with members of the *ras* family being most common. Presently identified oncogenes, however, are

**TABLE 17.1. Chromosomal Changes Leading To Oncogene Activation and the Human Malignancies Associated With Them**

Oncogene	Chromosomal Changes	Human Cancers
N-ras	Deletion (1)	Neuroblastoma
Blym	Deletion (1)	Neuroblastoma
fms	Deletion (5)	Acute nonlymphocytic leukemia
H-ras	Deletion (11)	Sarcoma
c-abl	Translocation (9-12)	Chronic myelogenous leukemia
c-myc	Translocation (8-14)	B-cell lymphoma
	Translocation (2-8)	Burkitt's lymphoma
hi-myc	Translocation (2-8)	Burkitt's lymphoma
raf	Translocation (3-8)	Parotid gland tumor
myb	Translocation (6-14)	Carcinoma
mas	Translocation (3-8)	Acute myelocytic leukemia
abl	Translocation (9-22)	Chronic myelogenous leukemia
sis	Translocation (9-22)	Chronic myelogenous leukemia
	Translocation (8-22)	Burkitt's lymphoma
N-myc	Gene amplification	Neuroblastoma
neu	Gene amplification	Breast carcinoma

associated with only a small proportion (10–15%) of human cancers and tend to be found more commonly with leukemias and lymphomas and less frequently with solid tumors. A critical feature of oncogenes is that they act in a *dominant* fashion. This means that the presence of a single copy of the activated oncogene in a cell is sufficient to produce the transformed phenotype, even in the presence of normal copy.

Some human leukemias and lymphomas appear to be caused by specific chromosomal translocations that lead to oncogene activation by essentially two mechanisms. One is transcriptional deregulation, in which the oncogene is overexpressed or incorrectly expressed, and the other is gene fusion.

There are many examples of the first mechanism, including follicular lymphoma, in which the *bcl-2* gene is deregulated by its juxtaposition to immunoglobulin enhancers. The other mechanism, gene fusion, was discovered in chronic myelogenous leukemia, in which the *abl* oncogene fuses with the *bcr* gene as a result of a symmetric translocation between chromosomes 9 and 22 (Fig. 17.3). Thus, essentially two major genetic mechanisms are involved in leukemogenesis and lymphomagenesis in hu-

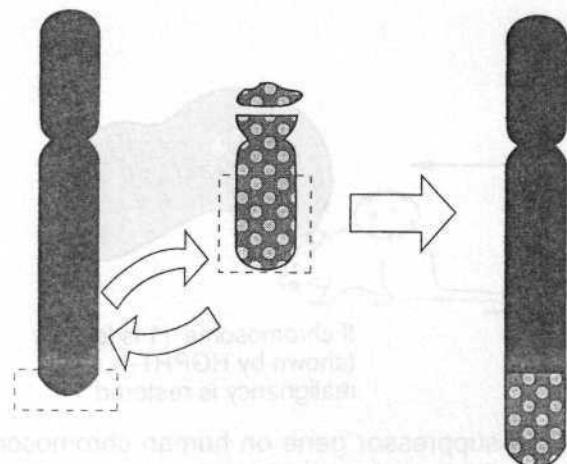
mans that lead to oncogene activation: enhancement of transcription and gene fusion.

### TUMOR-SUPPRESSOR GENES

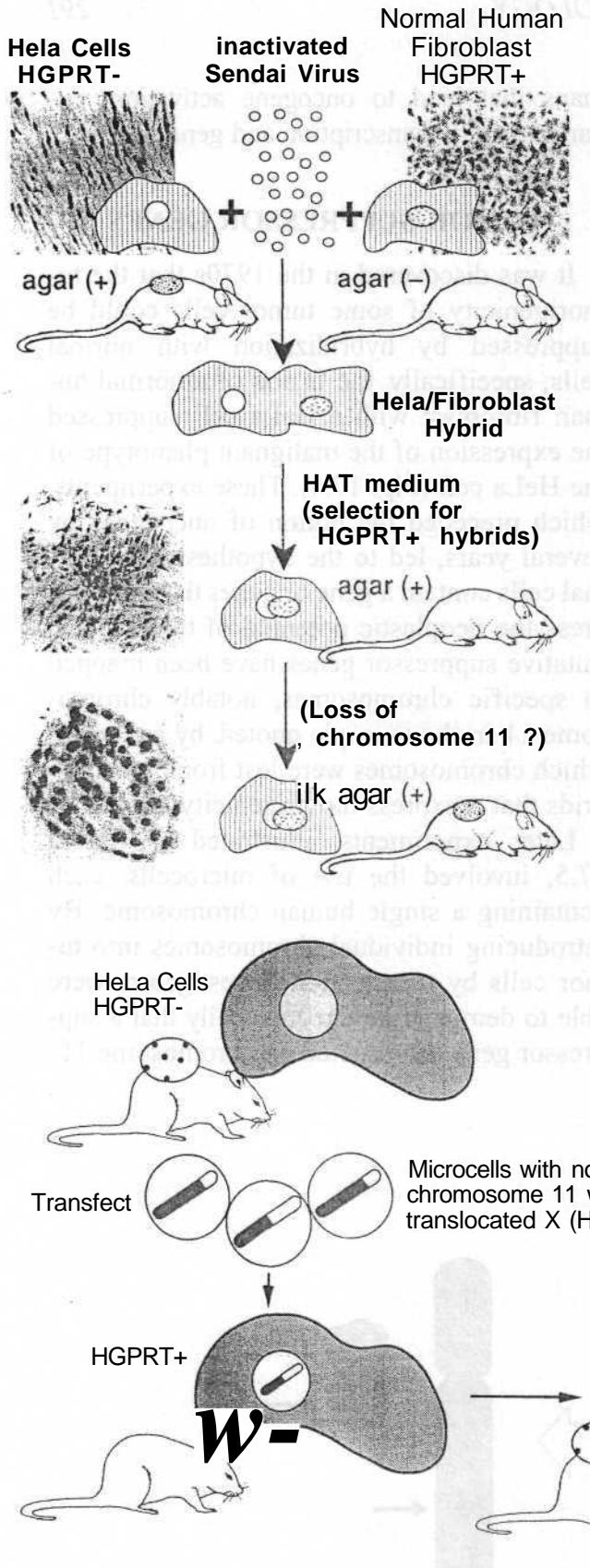
It was discovered in the 1970s that the tumorigenicity of some tumor cells could be suppressed by hybridization with normal cells; specifically, the fusion of a normal human fibroblast with a HeLa cell suppressed the expression of the malignant phenotype of the HeLa cell (Fig. 17.4). These experiments, which preceded the notion of oncogenes by several years, led to the hypothesis that normal cells contain a gene or genes that can suppress the neoplastic potential of tumor cells. Putative suppressor genes have been mapped to specific chromosomes, notably chromosome 11 in the example quoted, by analyzing which chromosomes were lost from those hybrids that reexpress tumorigenicity.

Later experiments, illustrated in Figure 17.5, involved the use of microcells, each containing a single human chromosome. By introducing individual chromosomes into tumor cells by transfection, investigators were able to demonstrate unequivocally that a suppressor gene was carried on chromosome 11.

Chromosome 9 Chromosome 22



**Figure 17.3.** How a symmetric translocation between chromosomes 9 and 22 brings together the *bcl-2* and *abl* genes to form a fusion gene, associated with over 90% of cases of chronic myelogenous leukemia (CML).



**Figure 17.4.** How hybrid cells can be used to demonstrate the way in which tumorigenicity may result from the loss of a suppressor gene. HeLa cells are tumorigenic; normal human fibroblasts are not. HeLa cells are transformed, as indicated by their ability to grow in soft agar, and tumorigenic, as evidenced by their ability to form tumors in immune-suppressed animals. Normal human fibroblasts do not show either property of malignancy. A hybrid formed by the fusion of a HeLa cell and a normal human fibroblast grows in soft agar but does not form tumors: The malignant phenotype of the HeLa cell is suppressed by the normal fibroblast. If, however, chromosome 11 from the normal fibroblast is lost, tumorigenicity is restored, indicating that the suppressor gene or antioncogene is located on that chromosome. (Illustrating the experiments of Stanbridge EJ: Suppression of malignancy in human cells. *Nature* 260:17-20, 1976.)

**Figure 17.5.** Illustrating the effect of the suppressor gene on human chromosome 11. HeLa cells are tumorigenic in nude mice. If a microcell is introduced containing chromosome 11 from a normal human fibroblast, the malignant phenotype of the HeLa cell is suppressed. If, during culture, chromosome 11 is lost, tumorigenicity is restored. The chromosome in the microcell is number 11 translocated with part of the X chromosome containing the HGPRT gene as a marker of the presence or absence of the chromosome in the HeLa cell. (Illustrating the experiments of Saxon PJ, et al.: *Mol Cell Biol* 5:140-146, 1985; and Saxon PJ, et al.: *EMBO J* 5:3461-3466, 1986.)

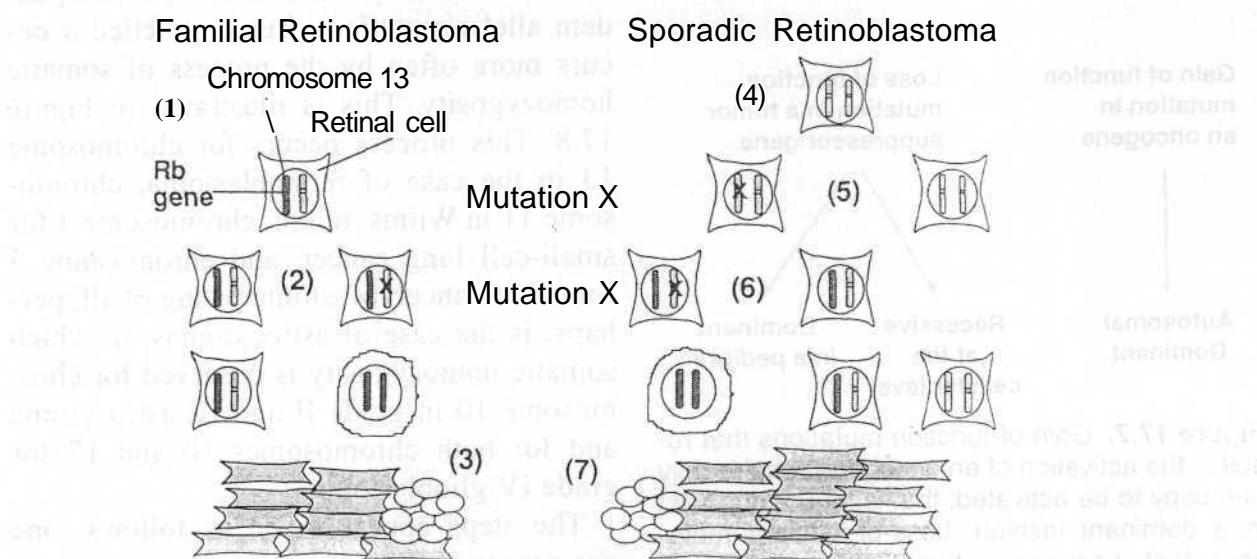
## THE PARADIGM OF RETINOBLASTOMA

Retinoblastoma is present in the human population in sporadic or familial forms. The sporadic form is relatively rare, occurring in one in 20,000 children. Malignancy results from the absence of a functional copy of the retinoblastoma (Rb) gene. Unlike oncogenes, which act in a dominant fashion, the Rb gene is recessive-acting, because its presence, even in a single copy, inhibits formation of the associated cancer. As early as 1971, Knudson postulated that all types of retinoblastoma involve two separate mutations that are carried by all retinoblastoma tumor cells. In the case of sporadic retinoblastoma, he argued that both mutations occur somatically, in the same retinal precursor cell, that is, after conception *in utero* or in early childhood, which then leads to a tumor in the absence of a functioning Rb gene. In the heritable form, in contrast, Knudson proposed that one of the two mutations is inherited from a parent and is therefore present at conception in all cells of the

developing retina, whereas the second mutation occurs spontaneously. The mechanisms of familial and sporadic retinoblastoma are illustrated in Figure 17.6. By the early 1980s the location of the suppressor gene involved in retinoblastoma was shown to be on the short arm of chromosome 13, and by the late 1980s the gene had been cloned and sequenced.

The Rb gene itself has now been implicated in several other human cancers, which indicates that it may play a generalized role in growth suppression in a variety of tissues. For example, patients who are cured of familial retinoblastoma are at increased risk of osteosarcoma, small-cell lung cancer, and breast cancer; although the loss of the Rb gene alone is sufficient for retinoblastoma, further changes are required for the development of these other tumors.

Wilms' tumor turns out to be more complex than retinoblastoma. Wilms' tumor is a kidney cancer in children that can occur in unilateral and early-onset bilateral forms (*i.e.*, sporadic or familial in the same way as retinoblas-



**Figure 17.6.** Rb mutations in familial and sporadic retinoblastoma. In familial retinoblastoma, one normal and one mutated Rb are inherited (1). Subsequent mutation in any retinal cell inactivates the remaining Rb (2), leading to loss of growth control in a clone of tumor cells (3). In sporadic retinoblastoma, two normal Rb are inherited (4). First, a mutation inactivates one copy of Rb (5). A subsequent mutation within the same retinal cell inactivates the remaining copy of Rb (6), leading to loss of growth control in a clone of tumor cells. (Illustrating the concepts proposed by Knudsen AG: Proc Natl Acad Sci USA 68:820-823, 1971.)

**TABLE 17.2.** Currently Identified Tumor Suppressor Genes

Suppressor Gene	Site	Associated Chromosome	Tumor
p-105 Rb	Nucleus	13q	Retinoblastoma
WT	Nucleus	3 different loci, 1p	Wilms' tumor
NFi	Cytoplasm	17q	Neurofibroma, sarcoma
FAP	?	17q	Familial adenomatous polyposis
p-53	Nucleus	17p	Breast cancer, small cell lung cancer, cervical cancer, bladder cancer
DCC	Cell surface	18q	Colon cancer

toma). Initially, one suppressor gene was mapped to chromosome 11p13, but a second genetic locus mapping to 11p15 has been implicated.

At the present time there are at least a dozen suppressor genes whose location and function are known, and more are likely to be discovered soon. The most common are listed in Table 17.2. The two most common and most intensively studied are the Rb gene and the p53 gene. Both of these are involved in control of the cell cycle and tumor differentiation. The distinction often is made that oncogenes are gain-of-function mutations, so that only one copy needs to be activated to result

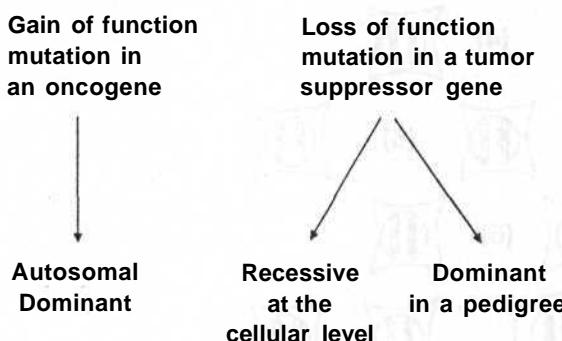
in the expression of the malignant phenotype, but tumor-suppressor genes are loss-of-function mutations: Both copies must be lost for the malignant phenotype to be expressed. In other words, oncogenes act in a dominant fashion whereas tumor-suppressor genes are recessive. Although this may be true at a cellular level, however, in viewing the pedigree of a family, loss of a tumor-suppressor gene may appear to be inherited as a dominant mutation. This is illustrated in Figure 17.7.

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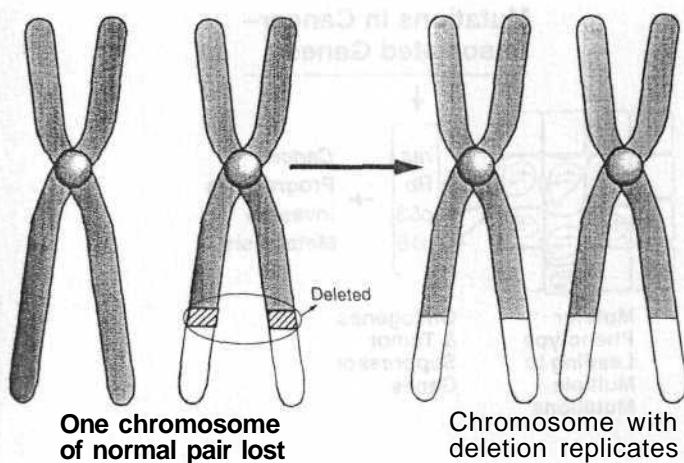
### SOMATIC HOMOZYGOSEITY

Both copies of a suppressor gene in the sporadic form of retinoblastoma and other solid tumors may result from two independent allelic mutations, but in practice it occurs more often by the process of somatic homozygosity. This is illustrated in Figure 17.8. This process occurs for chromosome 13 in the case of retinoblastoma, chromosome 11 in Wilms' tumor, chromosome 3 for small-cell lung cancer, and chromosome 5 for colon cancer. Most interesting of all, perhaps, is the case of astrocytomas, in which somatic homozygosity is observed for chromosome 10 in grade II and III astrocytoma and for both chromosomes 10 and 17 for grade IV glioblastoma.

The steps appear to be as follows: one chromosome of a pair is lost, a deletion occurs in the remaining chromosome, and the chromosome with deletion replicates. Instead of having one allele from each parent, the cell has both alleles from one parent, with a vital piece containing the tumor-suppressor gene missing.



**Figure 17.7.** Gain-of-function mutations that result in the activation of an oncogene require only one copy to be activated; that is, oncogenes act in a dominant fashion. Loss-of-function mutations that inactivate a tumor-suppressor gene require both copies to be inactivated for the malignant phenotype to be expressed; that is, tumor-suppressor genes act in a recessive fashion. This may be true at a cellular level; however, in viewing the pedigree of a cancer-prone family, the loss of a tumor-suppressor gene may appear to be inherited as a dominant mutation.



**Figure 17.8.** The process of somatic homozygosity. In a normal cell, there are two copies of each chromosome, one inherited from each parent. For a given suppressor gene to be inactivated, the copy must be lost from both chromosomes. This could, of course, occur by independent deletions from the two chromosomes, but in practice it is more common for a single deletion to occur in one chromosome while the second chromosome is lost completely. The remaining chromosome, with the deletion, then replicates. The cell is thus homozygous, rather than heterozygous, for that chromosome.

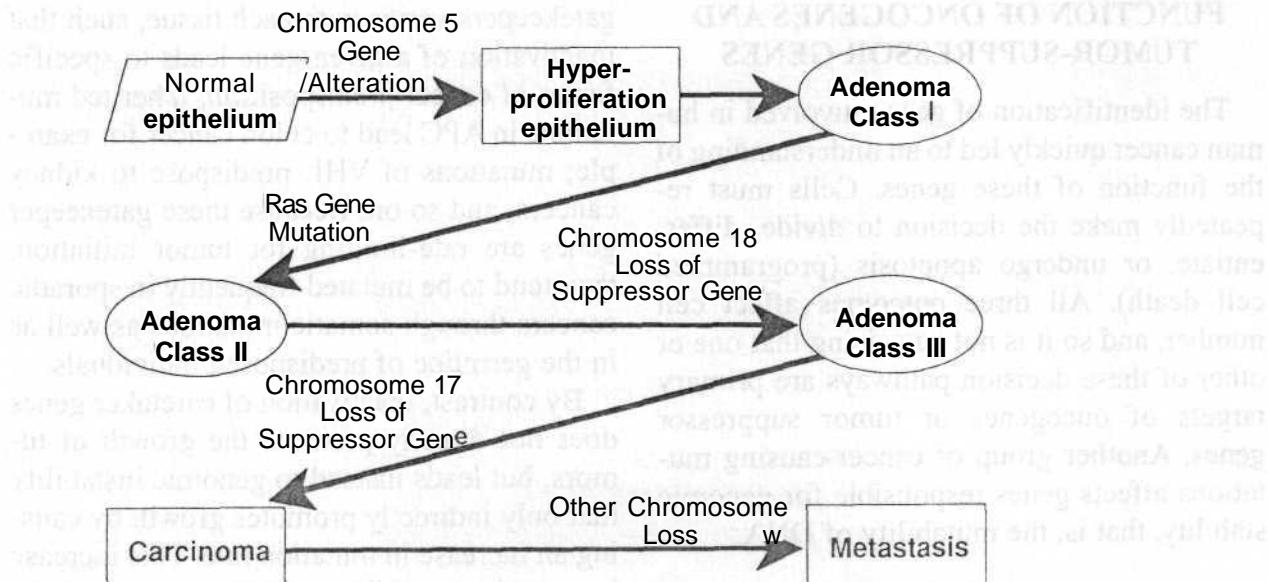
## MULTISTEP NATURE OF CANCER

Perhaps the most pervasive dogma in cancer research is that carcinogenesis is a multistage process. The implication is that there are a number of distinct events that may be separated in time. This idea is almost 70 years old and is exemplified by the skin-cancer experiments in mice that introduced the concepts of initiation, promotion, and progression as stages in tumor development.

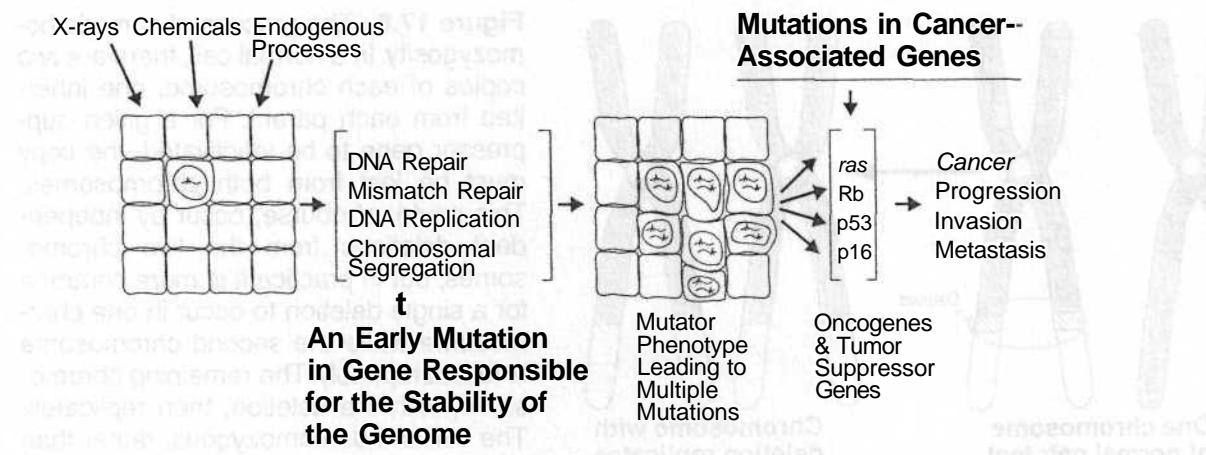
Genetic analysis of cells from solid tumors, too, suggests alterations, mutations, or dele-

tions in multiple signaling genes, either oncogenes or suppressor genes; 6 to 12 mutations have been suggested for the formation of a carcinoma. In the case of colorectal cancer, a model has been proposed that correlates a series of chromosomal and molecular events with the changes in the histopathology of normal epithelium during the multistage formation of colorectal cancer and metastatic carcinoma. This concept is illustrated in Figure 17.9.

A more general model of the series of events in carcinogenesis is shown in Figure 17.10. The first event, from whatever cause



**Figure 17.9.** Cancer has long been thought to be a multistep process and has been described with operational terms such as *initiation*, *promotion*, and *progression*. In at least one human malignancy, namely, colon cancer, the molecular events during the progress of the disease have been identified. (Based on the work of Vogelstein.)



**Figure 17.10.** Illustrating the multistep nature of carcinogenesis and the concept of the mutator phenotype. The first step in carcinogenesis by radiation or any other agent may be a mutation in one of the gene families responsible for the stability of the genome. This may be a DNA repair gene, a mismatch-repair gene, or a gene in a family as yet unidentified. This leads to the mutator phenotype, with multiple mutations possible in both oncogenes and tumor-suppressor genes. This leads to a series of steps that result in an invasive metastatic cancer. Not all the same mutations need to be present in every case.

(including ionizing radiation), causes a mutation in a gene in one of the families responsible for the stability of the genome. This leads to a mutator phenotype, so that with many cells dividing, multiple mutations are likely in cancer-associated genes, both oncogenes and tumor-suppressor genes. This in turn leads to progression of the cancer and ultimately its invasive and metastatic properties.

### FUNCTION OF ONCOGENES AND TUMOR-SUPPRESSOR GENES

The identification of genes involved in human cancer quickly led to an understanding of the function of these genes. Cells must repeatedly make the decision to divide, differentiate, or undergo apoptosis (programmed cell death). All three outcomes affect cell number, and so it is not surprising that one or other of these decision pathways are primary targets of oncogenes or tumor suppressor genes. Another group of cancer-causing mutations affects genes responsible for genomic stability, that is, the mutability of DNA.

### GATEKEEPERS AND CARETAKERS

It appears that most tumor-suppressor genes can be broadly divided into two classes that have been called "gatekeepers" and

"caretakers." Gatekeepers are genes that directly regulate the growth of tumors by inhibiting cell division or promoting cell death. The function of these genes, therefore, is rate-limiting for tumor growth; both alleles (maternal and paternal) must be lost or inactivated for a tumor to develop. Predisposed individuals inherit one damaged copy of such a gene and so require only one additional mutation for tumor initiation. The identity of gatekeepers varies with each tissue, such that inactivation of a given gene leads to specific forms of cancer predisposition; inherited mutations in APC lead to colon cancer for example; mutations of VHL predispose to kidney cancers, and so on. Because these gatekeeper genes are rate-limiting for tumor initiation, they tend to be mutated frequently in sporadic cancers through somatic mutations as well as in the germline of predisposed individuals.

By contrast, inactivation of caretaker genes does not directly promote the growth of tumors, but leads instead to genomic instability that only indirectly promotes growth by causing an increase in mutation rate. This increase in genetic instability can accelerate greatly the development of cancers, especially those that require numerous mutations for their full development. Colon cancer is a good example. The targets of the accelerated mutation

rate that occurs in cells with defective caretakers are the gatekeeper tumor-suppressor genes, oncogenes, or both.

This description is almost certainly oversimplified. Some genes clearly defy being put into either category. It is an interesting and useful concept, however, that no doubt will be refined in the future.

### MISMATCH REPAIR

Interest in **mismatch-repair genes** heightened with the discovery that they were responsible for the mutator phenotype associated with a predisposition for hereditary nonpolyposis colon cancer (HNPCC) and possibly other familial cancers. The initial clue to this novel molecular mechanism was the discovery of deletions of long monotonic (dA-dT) runs in a subset of human colon cancers. Soon afterwards, insertions or deletions at mono-, di-, and trinucleotide repeat sequences were discovered in subsets of colon tumors as well as in a majority of colon cancers from individuals with HNPCC. This phenotype also has been detected in several other types of human malignancies, especially those associated with type 2 Lynch syndrome. These various investigations culminated in the identification and cloning of the human hMSH 2 gene, which maps to a locus linked to HNPCC on chromosome 2p21-22 and whose homologs in *Saccharomyces cerevisiae* and *Escherichia coli* are involved in the process of DNA mismatch repair.

The primary function of mismatch-repair genes in *E. coli* appears to be to scan the genome as it replicates and to spot errors of mismatch as the DNA is replicated, that is, as the new strand is laid down using the stable methylated strand as a template. A growing number of human genes has been associated with HNPCC by means of linkage analysis and studies of mutational mapping. Table 17.3 is the current listing of these human mismatch-repair genes, together with their chromosomal location. The list may not be complete. The mismatch-repair process in yeast and bacteria involves a large number of proteins, and so it is likely that additional causes of HNPCC remain to be uncovered.

TABLE 17.3. *Human Mismatch Repair Genes*

Gene	Chromosomal Location	Germline Mutations in HNPCC Cases (Reported Family Studies), %
hMSH2	2p21-22 (2p16?)	60
hMLH1	3p21	30
hPMS1	2q31-33	4
hPMS2	7p21	4
GTBP	2p16(2p21-22?)	0

Cells with defective or nonfunctioning mismatch-repair genes can be identified by two quite different techniques:

1. The use of a selectable reporter system that inserts an exogenous long repeat sequence into the cells in question and measures the mutation rate in it
2. Measuring the mutation rate in one or more of the many endogenous repeat sequences that already exist in every human cell: the so-called microsatellite instability assay

Both techniques have strengths and weaknesses and both are far from perfect.

Microsatellite instability appears to be a factor of some importance in a wide variety of human tumors.

### TELOMERES AND CANCER

An important link between genomic stability and cancer is the telomere hypothesis. Telomeres cap and protect the ends of chromosomes. Mammalian telomeres consist of long arrays of the repeat sequence TTAGGG that range in length anywhere from 1.5 to 150 kilobases. Each time a normal somatic cell divides, the terminal end of the telomere is lost; successive divisions lead to progressive shortening, and after 40 to 60 divisions vital DNA sequences are lost. At this point the cell cannot divide further and undergoes senescence. Telomere length has been described as the "molecular clock," because it shortens with age in somatic tissue cells during adult life. Stem cells in self-renewing tissues, and cancer cells in particular, avoid this process of aging by activating the enzyme telomerase.

Telomerase is a reverse transcriptase that polymerizes TTAGGG repeats to offset the degradation of chromosome ends that occurs with successive cell divisions; in this way the cell becomes immortal. Because immortality and uncontrolled growth are the hallmarks of cancer cells, the activation of telomerase would appear to be a vital step in carcinogenesis. In tissue culture, immortalization of cells, that is, cells that pass through a "crisis" and continue to be able to divide beyond the normal limit, is associated with telomere stabilization and activity of telomerase. Telomerase activity also has been detected in cells from carcinomas. The question remains: Is immortalization or carcinogenesis intimately associated with telomerase expression? To complicate the picture, however, tumor cells have been identified recently with stable telomere length but undetectable telomerase activity.

### SIGNAL TRANSDUCTION

An elaborate system of biochemical communication networks exists in eukaryotic cells to provide information to the nucleus regarding the environment outside the cell. *Signal transduction* is the term used to describe the flow of information from the cell's outer membrane, through the cytoplasm, and into the nucleus. Multiple signal-transduction cascades can operate in tandem, with variable amounts of "cross-talk" between them. A weak signal is capable of eliciting a powerful and pervasive response because it is amplified by biologic processes, in much the same way as a radio signal is amplified by an electronic device.

Signals, in the form of cytokines, low molecular weight hormones, growth factors, and other proteins that arrive at the outer plasma membrane can enter the cell *via* two principal mechanisms:

1. Cell-surface receptors that span the plasma membrane and selectively bind individual cytokines and hormones. These receptor molecules transduce a signal from their extracellular domain to

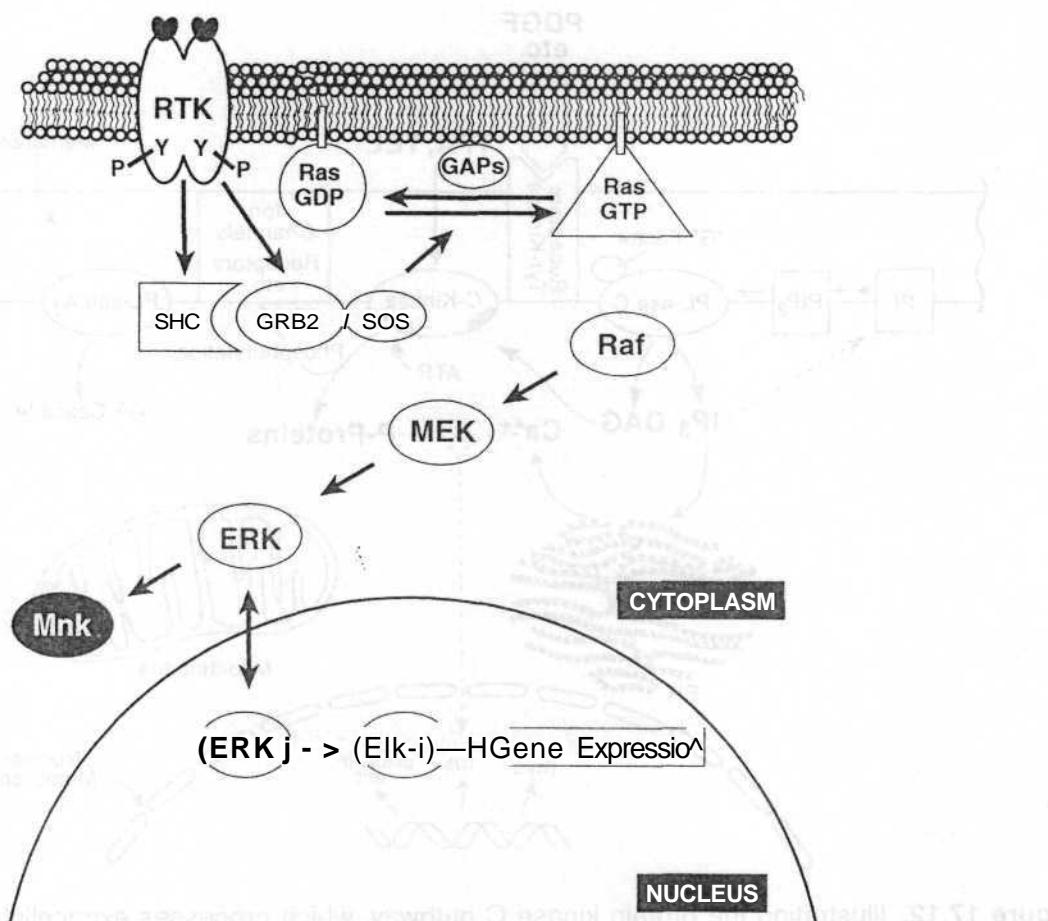
their cytoplasmic domain on the inner surface of the plasma membrane. This frequently leads to the activation of an enzyme, such as, for example, a protein kinase.

2. Some environmental agents can migrate across the plasma membrane and deliver signals directly to the cytoplasm, at which they bind to specific receptor molecules.

In either case, a biochemical cascade ultimately allows the signal to reach its destination, which is the nucleus, and alter cellular behavior. These signal-transduction processes are characterized by signaling molecules that are bound together by protein-protein interactions, and by the production of second messenger molecules.

One important signal-transduction pathway involves the GTP binding proteins, including the *ras* family. These serve as molecular switches for various physiologic processes that regulate cell growth. *Ras* is a key regulator of cell growth in all eukaryotic cells. It has been estimated that 30% of all human tumors contain an activating mutation in *ras*. The frequency varies depending on tumor type, with the highest frequencies seen in lung, colon, thyroid, and pancreatic carcinomas. Three alleles of *ras* have been described: Ha-, Ki-, and N-*ras*. Growth factors (such as platelet-derived growth factor, epidermal growth factor, and fibroblast growth factor) bind to receptors that have an extracellular ligand-binding domain, span the membrane, and trigger a tyrosine kinase in the cytoplasm. This is illustrated in a simplified form in Figure 17.11. *Ras* must target at least three different pathways for transformation. The corruption of signaling pathways that lie downstream of *ras* is a recurring theme in the initiation and progression of human malignancies. If cells are transformed by *ras*, DNA synthesis is stimulated, and there are alterations in the morphology of the cell.

Another example of a signal-transduction pathway, namely protein kinase C (PKC), is illustrated diagrammatically in Figure 17.12. PKC is known to be the cellular receptor for

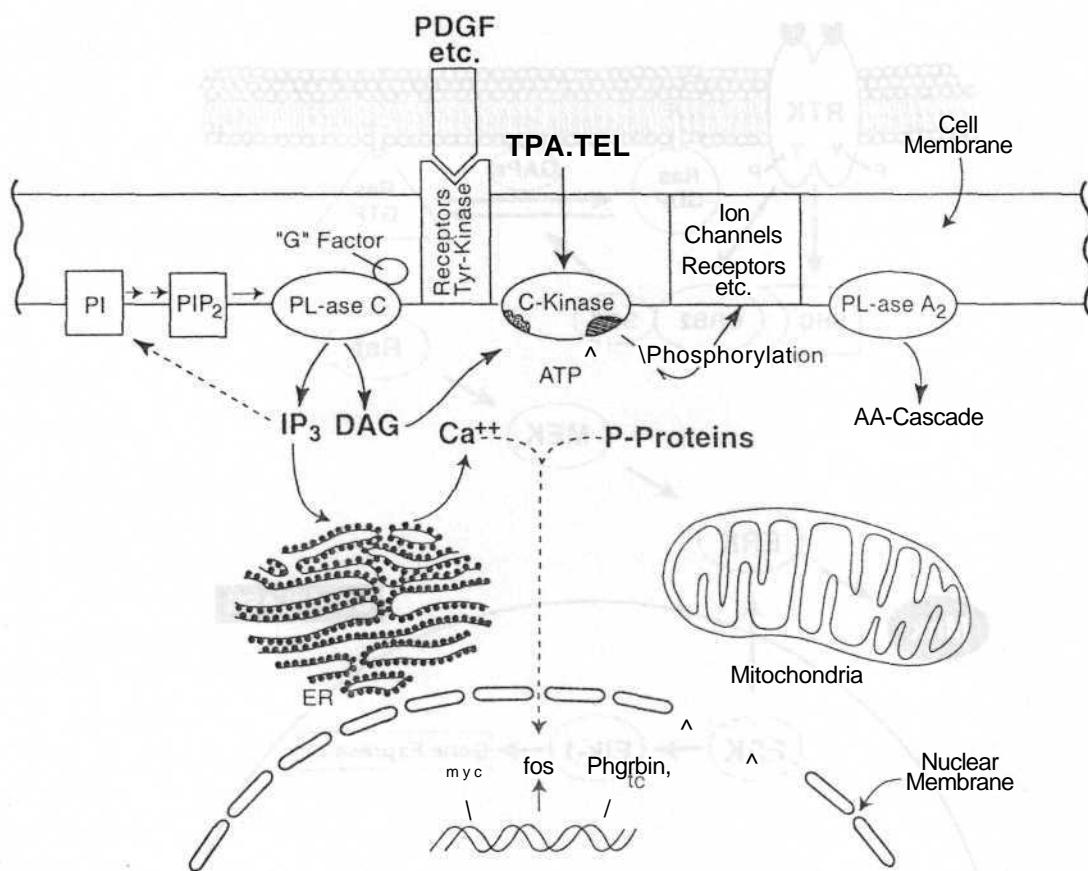


**Figure 17.11.** Ras mediates its effect on cellular proliferation, at least in part, by the activation of a cascade of kinases. Ras is a GDP/GTP-regulated binary switch that resides at the inner surface of the plasma membrane and acts to relay extracellular ligand-stimulated signals to cytoplasmic signaling cascades. A linear pathway in which ras functions downstream of receptor tyrosine kinases and upstream of a cascade of serine-threonine kinases provides a complete link between the cell surface and the nucleus. Activated ERKs can translocate into the nucleus to phosphorylate and activate transcription factors, such as Elk-1. Activated ERKs also phosphorylate substrates in the cytoplasm, including the Mnk kinase, and thus contribute to translation initiation of mRNAs with structured 5'-untranslated regions. This is an oversimplified illustration, because there are at least three signaling pathways that lie downstream of ras. (Adapted from Vojtek AB, Der CJ: Increasing complexity of the ras signaling pathway. *J Biol Chem* 273:19925-19928, 1998, with permission.)

the phorbol esters family of tumor promoters. In addition, PKC is known to mediate events along signaling pathways shared by growth factors, protooncogenes and their activated counterparts, and early-response genes. In some mammalian cell-signaling pathways, PKC lies downstream of specific growth factors and hormone receptors.

In general, signal-transduction cascades are critically dependent on serial phosphorylation-dephosphorylation reactions catalyzed by kinases and phosphates to regulate and ac-

tivate their respective activities. Protein phosphorylation is the most common posttranslational modification and plays a key role in regulating both cell-cycle events and signal-transduction cascades. Kinases add phosphates to proteins by transferring phosphate groups from ATP to hydroxyl groups on amino acids; phosphatases remove the phosphate groups. Protein kinases and phosphatases are divided into three groups based on the amino acids they phosphorylate and dephosphorylate. One class recognizes serine



**Figure 17.12.** Illustrating the protein kinase C pathway, which processes extracellular signals and transmits them to the nucleus. Extracellular growth factors (such as PDF) activate a receptor, which in turn activates a G protein that in turn activates the phospholipase C. In less than a second, the enzyme cleaves PIP<sub>2</sub> to generate two products, inositol triphosphate and diacylglycerol. IP<sub>3</sub> is a small water-soluble molecule that releases Ca<sup>2+</sup> from the calcium-sequestering compartment, such as the endoplasmic reticulum. The enzyme activated by diacylglycerol is protein kinase C, called such because it is Ca<sup>2+</sup> dependent. Protein kinase C phosphorylates specific serine and threonine residues on cellular proteins in addition to activating the plasma membrane Na<sup>+</sup>H<sup>+</sup> exchanger that controls intracellular pH. Also, the calcium enters the nucleus and activates the transcription of growth-regulating genes, such as *myc* and *fos*.

and threonine residues, another recognizes tyrosine residues, and a small group recognizes both serine-threonine and tyrosine residues. Kinases and phosphatases may have a single substrate or multiple substrates. Phosphorylation or dephosphorylation of a given amino acid can have either stimulatory or inhibitory effects on the protein's enzymatic activity or ability to bind certain proteins or to specific DNA sequences.

A single protein can have multiple sites of phosphorylation that act as substrates to a number of different kinases, resulting in either activating or inhibitory changes in the protein. There are many examples of this including the case of p53 and CDC2.

## RADIATION-INDUCED SIGNAL TRANSDUCTION

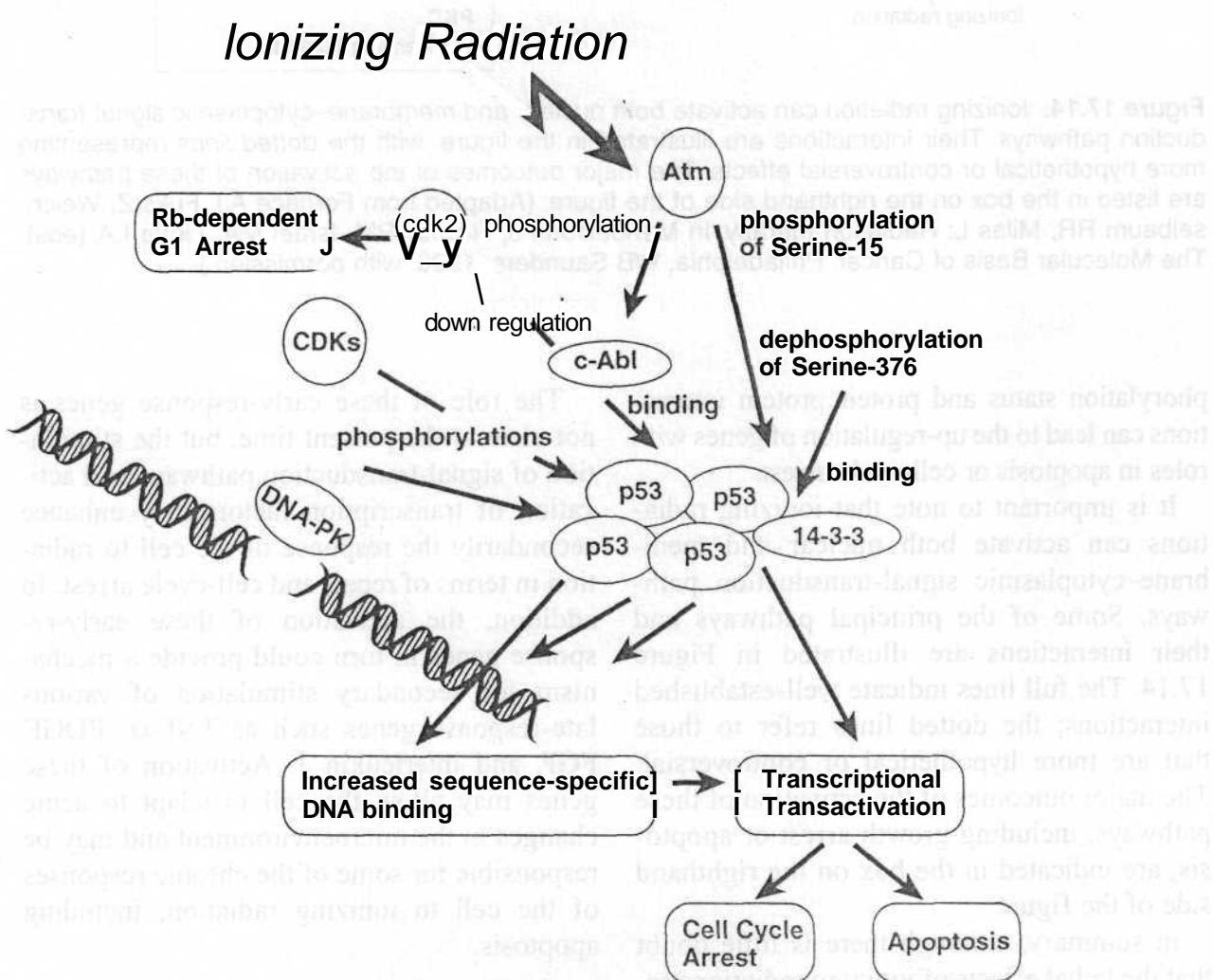
Ionizing radiation can act to regulate the expression of a number of genes including *c-fos*, *c-jun*, and *c-myc*, as well as those involved in cell-cycle control.

The activation of early-response genes by ionizing radiation suggests that radiation in some way mimics the mitogenic activation of quiescent cells. Evidence for the importance of such effects is that the overexpression of protooncogenes such as *c-ras* and *c-raf*, whose products are intermediates in the pathways of mitogenic signal transduction, is associated with radiation resistance. The serum response

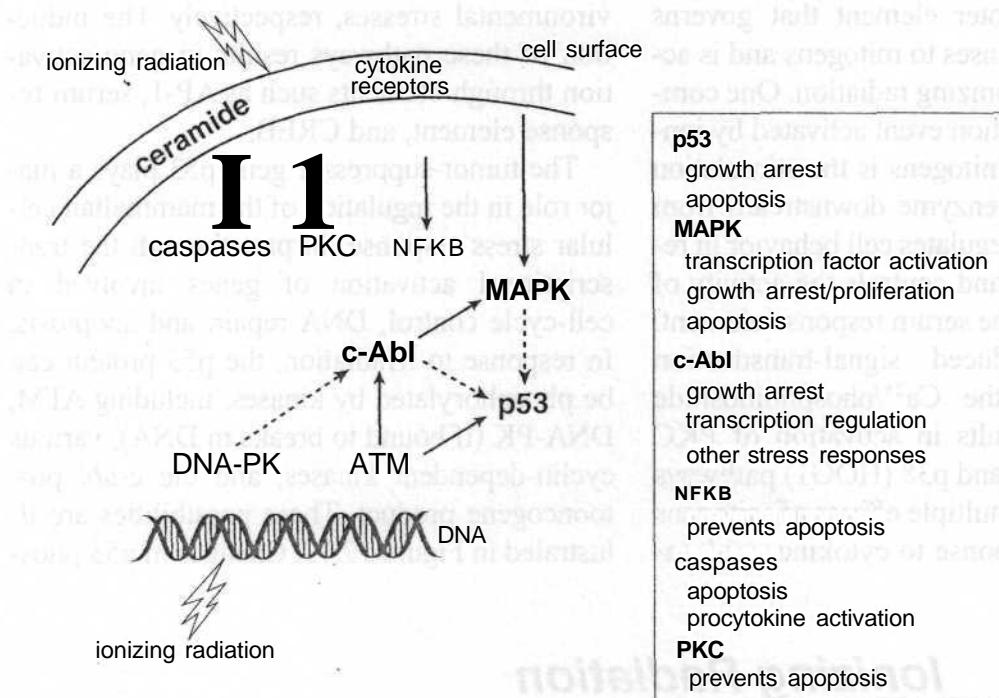
element is a promoter element that governs transcriptional responses to mitogens and is activated strongly by ionizing radiation. One common signal-transduction event activated by ionizing radiation and mitogens is the stimulation of MAP-kinase, an enzyme downstream from *c-ras* and *c-ra*/that regulates cell behavior in response to mitogens and controls the activity of genes regulated by the serum response element. Other radiation-induced signal-transduction pathways include the  $\text{Ca}^{2+}$ /phosphoinositide pathway, which results in activation of PKC and the JNK (SAP) and p38 (H0G1) pathways that coordinate the multiple effects of mitogens and the cellular response to cytokines and en-

vironmental stresses, respectively. The induction of these pathways results in gene activation through elements such as AP-1, serum response element, and CR $\epsilon$ B.

The tumor-suppressor gene p53 plays a major role in the regulation of the mammalian cellular stress response, in part through the transcriptional activation of genes involved in cell-cycle control, DNA repair, and apoptosis. In response to irradiation, the p53 protein can be phosphorylated by kinases, including ATM, DNA-PK (if bound to breaks in DNA), various cyclin-dependent kinases, and the *c-abl* protooncogene product. These possibilities are illustrated in Figure 17.13. Changes in p53 phos-



**Figure 17.13.** The role of p53 in transduction of the signal from radiation damage. The transcriptional activity of p53 is modulated in response to DNA damage by the activity of a number of kinases (including ATM and DNAPk), as well as by protein:protein interactions, resulting in either cell-cycle arrest or apoptosis. (Adapted from Amundsen SA, Myers TG, Fornace AJ: Roles of p53 in growth arrest and apoptosis: Putting on the brakes after genotoxic stress. *Oncogene* 17:3287-3299, 1998, with permission.)



**Figure 17.14.** Ionizing radiation can activate both nuclear and membrane-cytoplasmic signal transduction pathways. Their interactions are illustrated in the figure, with the dotted lines representing more hypothetical or controversial effects. The major outcomes of the activation of these pathways are listed in the box on the righthand side of the figure. (Adapted from Fornace AJ, Fuks Z, Weichselbaum RR, Mrias L: Radiation therapy. In Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds): *The Molecular Basis of Cancer*. Philadelphia, WB Saunders, 1998, with permission.)

phorylation status and protein:protein interactions can lead to the up-regulation of genes with roles in apoptosis or cell-cycle arrest.

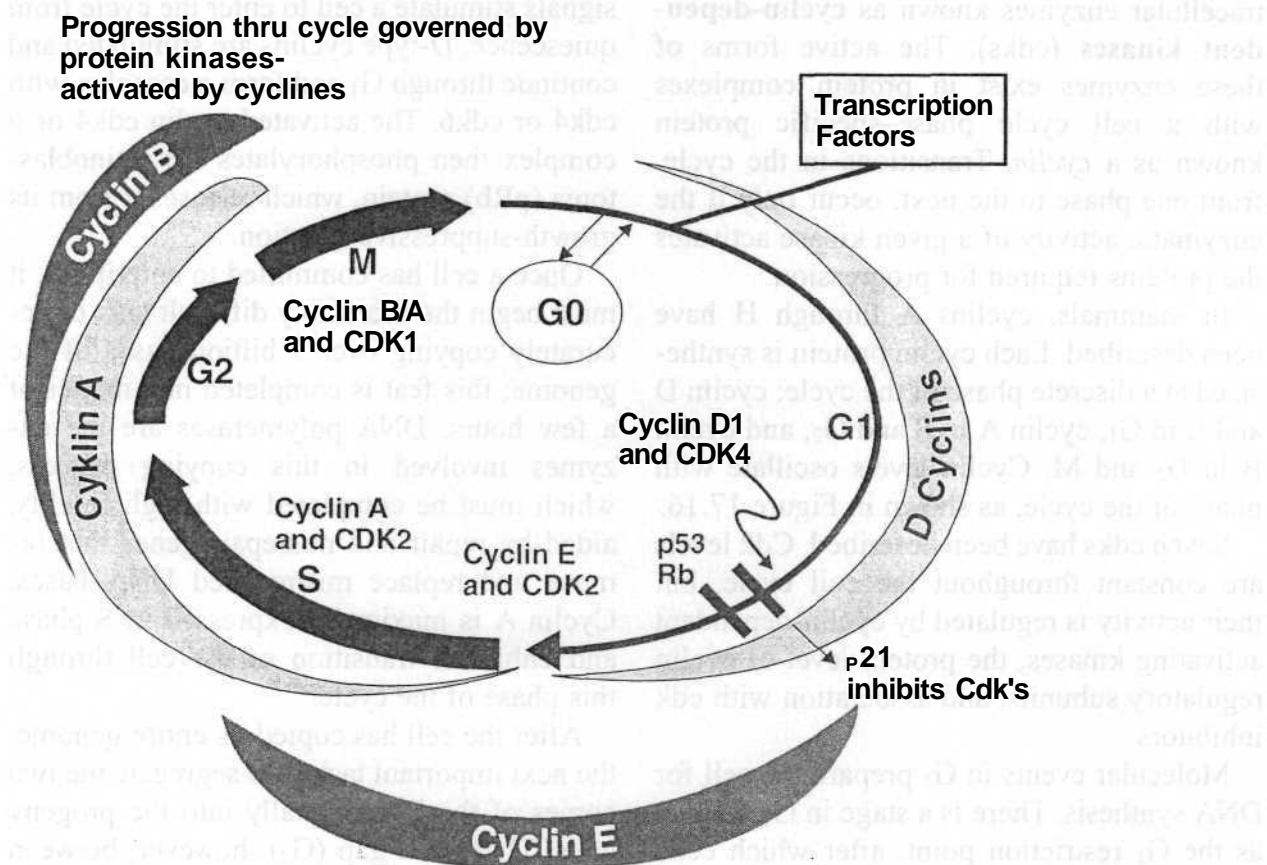
It is important to note that ionizing radiations can activate both nuclear and membrane-cytoplasmic signal-transduction pathways. Some of the principal pathways and their interactions are illustrated in Figure 17.14. The full lines indicate well-established interactions; the dotted lines refer to those that are more hypothetical or controversial. The major outcomes of the activation of these pathways, including growth arrest or apoptosis, are indicated in the box on the righthand side of the figure.

In summary, although there is little doubt that the lethal effects of ionizing radiations result from extensive DNA damage leading to chromosomal aberrations, much lower doses of radiation can stimulate signal-transduction pathways, resulting in the activation of downstream transcription factors.

The role of these early-response genes is not clear at the present time, but the stimulation of signal-transduction pathways and activation of transcription factors may enhance secondarily the response of the cell to radiation in terms of repair and cell-cycle arrest. In addition, the activation of these early-response genes in turn could provide a mechanism for secondary stimulation of various late-response genes such as TNF- $\alpha$ , PDGF, FGF, and interleukin 1. Activation of these genes may allow the cell to adapt to acute changes in the microenvironment and may be responsible for some of the chronic responses of the cell to ionizing radiation, including apoptosis.

## THE CELL CYCLE

The ability of cells to produce exact, accurate copies of themselves is essential to the continuance of life; it is accomplished



**Figure 17.15.** The current concept of the cell cycle and its regulation by protein kinases, activated by cyclins.

through highly organized processes, well conserved through evolution. Lack of fidelity in cellular reproduction as manifested by DNA and chromosome alterations is a hallmark of cancer.

The only event in the cell cycle that can be identified with a simple light microscope is the condensation of the chromosomes during mitosis; this was observed in the late 19th century. Using autoradiography, Howard and Pelc in the early 1950s divided up the cell cycle by showing that DNA was synthesized only during a discrete time interval, which they called *S phase*. Between mitosis and the *S* phase was the "first gap in activity" (*G<sub>i</sub>*), and between *S* phase and the next mitosis the "second gap in activity" (*G<sub>2</sub>*).

They also showed that it was in these gaps that radiation has effects on cell-cycle progression, because even in their early studies it was obvious that cells arrest cell-cycle pro-

gression after low-dose radiation damage not in *S* or *M* but in either *G<sub>i</sub>* or *G<sub>2</sub>*. It was subsequently recognized that these arrests also were related to the process of malignancy, because primary cells would arrest in both *G<sub>i</sub>* and *G<sub>2</sub>*, but tumor cells often would show only the *G<sub>2</sub>* arrest point. The breakthroughs in understanding these events and the nature of the cell cycle itself came with the discovery of the cyclins, the cyclin-dependent kinases and the cyclin kinase inhibitors, and with the elaboration by Weinert and Hartwell of the concept of cell-cycle checkpoints. The current concept of the cell cycle and its regulation is illustrated in Figure 17.15.

### CYCLINS AND KINASES

Regulation of the complex processes that occur as a cell passes through the cycle is a result of a series of changes in the activity of in-

extracellular enzymes known as **cyclin-dependent kinases** (cdks). The active forms of these enzymes exist in protein complexes with a cell cycle phase-specific protein known as a *cyclin*. Transitions in the cycle, from one phase to the next, occur only if the enzymatic activity of a given kinase activates the proteins required for progression.

In mammals, cyclins A through H have been described. Each cyclin protein is synthesized at a discrete phase of the cycle: cyclin D and E in G<sub>i</sub>, cyclin A in S and G<sub>2</sub>, and cyclin B in G<sub>2</sub> and M. Cyclin levels oscillate with phase of the cycle, as shown in Figure 17.16.

Seven cdks have been described. Cdk levels are constant throughout the cell cycle, but their activity is regulated by cyclin-dependent activating kinases, the protein level of cyclin regulatory subunits, and association with cdk inhibitors.

Molecular events in G<sub>i</sub> prepare the cell for DNA synthesis. There is a stage in G<sub>i</sub>, known as the G<sub>i</sub> restriction point, after which cells are committed to enter the S phase and no longer respond to growth conditions. Prior to this point, cells may take several routes: They may progress, differentiate, senesce, or die, depending on external signals. Key players in the G<sub>i</sub> restriction point include the protein of the Rb gene, D-type cyclins, and cdk4 and cdk6, as well as cdk inhibitors. If extracellular

signals stimulate a cell to enter the cycle from quiescence, D-type cyclins are stimulated and continue through G<sub>i</sub> and form a complex with cdk4 or cdk6. The activated cyclin cdk4 or 6 complex then phosphorylates the retinoblastoma (pRb) protein, which releases it from its growth-suppressive function.

Once a cell has committed to entering S, it must begin the incredibly difficult task of accurately copying over 3 billion bases of the genome; this feat is completed in a matter of a few hours. DNA polymerases are the enzymes involved in this copying process, which must be completed with high fidelity, aided by repair and misrepair genes that remove and replace mismatched DNA bases. Cyclin A is maximally expressed in S phase and enhances transition of the cell through this phase of the cycle.

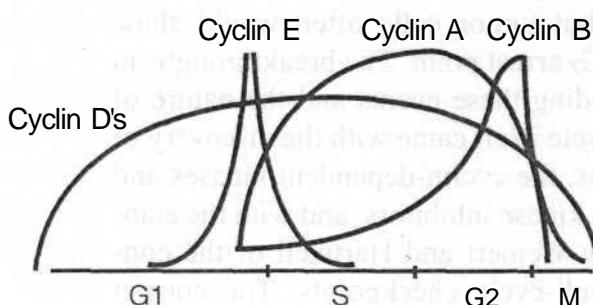
After the cell has copied its entire genome, the next important task is to segregate the two copies of the DNA equally into the progeny cells. There is a gap (G<sub>2</sub>), however, between the end of all detectable DNA synthesis and the beginning of cell division, at which the process of condensing and segregating the chromosomes begins. Events during this period are controlled by cdk activity analogous to that occurring at the G<sub>i</sub> to S transition, but this time it is a complex of cyclins B and A with cdk 1.

Although the cell is progressing through this complicated process of DNA replication and division, it must respond constantly to extracellular signals concerning the nutrient status, cell-to-cell contact, and so forth that arrive at the nucleus through one or another signal-transduction pathway.

#### CHECKPOINT GENES

For obvious reasons, events in the cell cycle must take place in a specific order, and it is the function of a number of checkpoint genes to ensure that the initiation of late events is delayed until earlier events are complete.

There are three principal places in the cell cycle at which checkpoints function:



**Figure 17.16.** Progression through the cell cycle from one phase to the next is governed by protein kinases, activated by cyclins. In mammals, cyclins A through H have been described; each cyclin protein is synthesized at a discrete phase of the cell cycle. Cyclin levels oscillate with phase of cycle, as shown schematically in this figure.

1. G<sub>1</sub>/S checkpoint
2. S-phase checkpoint
3. G<sub>2</sub>/M checkpoint

If DNA is damaged, normal cells stop progressing through the cycle and are arrested at one of these checkpoints, depending on their positions in the cell cycle at the time at which the damage occurs.

Cells with damaged DNA in G<sub>1</sub> avoid replicating that damage by arresting at the G<sub>1</sub>/S interface or, if they have already passed the point of no return, by transiently arresting in the S phase. Avoiding the replication of damaged DNA, and allowing time for repair, limits cell death and heritable mutations. The tumor-suppressor gene p53 is critical in the pathway that leads to G<sub>1</sub> arrest. DNA damage initiates a chain of events: First p53 protein levels are increased, and then activated p53 enhances p21 WAF1/CIP1 gene expression, the increased expression of which results in the inhibition of G<sub>1</sub> cyclin/cdk's, which prevents phosphorylation of pRb and progression from G<sub>1</sub> into S. Mutations in p53 (which are present in so many human tumors) clearly compromise this checkpoint function. The G<sub>1</sub>/S checkpoint is the most completely understood of the three listed above. Less is known about the control of the S-phase checkpoint.

The arrest of cells in G<sub>2</sub> following DNA damage is observed readily in mammalian cells and has been studied by radiation biologists for decades, since before checkpoints were understood at the molecular level. The arrest occurs after the up-regulation of cyclin A, but before the up-regulation of cyclin B. The function of this checkpoint in normal cells is to prevent cells with damaged chromosomes from attempting the complex process of mitosis; they are arrested in G<sub>2</sub> to allow DNA repair to be completed. It follows that cells lacking the G<sub>2</sub> checkpoint are radiosensitive, because they try to divide although their chromosomes are damaged. The controlling genes for this checkpoint have been identified in yeast, but not in mammalian cells, although the ATM gene is a likely participant.

The hallmark of cancer is a lack of the ability to respond to signals that normally would cause the cell to stop progressing through the cycle and dividing.

Of the tumor-suppressor genes discovered so far in human cancers, only pRb and p53 appear to be directly implicated in cell-cycle control; there is clearly much more to be learned on this topic.

## CANCER GENETICS

The link between cancer risk and genetic factors varies among tumor types. Up to 30% of rare childhood cancers occur in individuals who are predisposed, whereas only about 5 to 10% of common adult cancers occur in an hereditary setting. Many instances of cancer predisposition are characterized by a distinctive pattern involving several different types of cancer in a number of different organs, sometimes accompanied by unusual physical features. In general, a family history of cancer, early age of onset, with multiple primary tumors, strongly suggest that the cause is a genetic disorder.

There are four ways in which germ-line mutations can result in a predisposition to cancer:

1. The majority of cases of hereditary cancer predisposition can be traced to germline mutations in tumor suppressor genes. Indeed, tumor-suppressor genes were first discovered through a study of familial predisposition to retinoblastoma,
2. The only disorders for which there is evidence of germline activation of an oncogene is multiple endocrine neoplasia. Medullary thyroid carcinoma and pheochromocytoma are features of these autosomal dominant syndromes. Physical abnormalities often accompany the malignancy.
3. Any syndrome that includes DNA repair defects is likely to increase the probability of mutations in genes that lead to cancer. Xeroderma pigmentosum, ataxia telangiectasia, Bloom syndrome, and Fanconi's

anemia are examples of relatively rare autosomal recessive syndromes with faulty DNA repair or replication. A particularly interesting member of this class is HNPCC, which is caused by a mutation in one of five mismatch-repair genes. A mutation in one of these genes leads to a general instability known as the *mutator phenotype*. This was described previously in this chapter.

4. A lesser understood category of hereditary disorders predisposes to cancer through unusual sensitivity to common carcinogens. For example, lung cancer usually is considered to have only a small genetic component, but genetic variations in the metabolism of the carcinogens in tobacco smoke may be important factors determining who develops a malignancy. Genetic variation may also have an influence on susceptibility to hepatic cellular carcinoma caused by aflatoxin.

A number of hereditary disorders that are known to predispose to cancer are listed here; they are at various stages of understanding at the molecular level. Retinoblastoma displays the clearest pattern of a familial and sporadic form, which can be understood readily and explained. Some researchers speculate that when all of the information is in, all common cancers will be seen to have familial and sporadic components—but the genetics of common cancers may be more complicated than for retinoblastoma, and the pattern far less obvious.

Hereditary retinoblastoma  
Hereditary Wilms' tumor  
Xeroderma pigmentosum  
Li-Fraumeni syndrome

Neurofibromatosis types 1 and 2  
Neurofibromatosis type 2  
Nevoid basal cell carcinoma syndrome  
Familial adenomatous polyposis coli  
BRCA-1: Familial female breast and ovarian cancer  
BRCA-2: Familial female and male breast cancer

### ATAXIA TELANGIECTASIA AND CANCER

There are a number of genetically transmitted repair deficiencies in humans. One of the most well-known and most extensively studied is **ataxia telangiectasia** (AT).

Homozygotes have distinctive neurologic disorders and oculocutaneous telangiectasia. It has long been known that the AT gene predisposes those carrying it to cancer with a risk 61 to 184 times higher than the general population. The AT gene also is associated with an unusual sensitivity to ionizing radiation. Homozygotic persons suffer devastating tissue necrosis if exposed to conventional radiotherapy, and cultured cells from such people are more sensitive than control cells to x-rays by a factor of about 3. What has long been debated is whether heterozygotes, persons carrying one copy of the mutated gene, are likewise radiosensitive and susceptible to cancer. Although on average cells cultured from AT heterozygotes are more sensitive to x- or y-rays, there is so much overlap with the wide range of sensitivities of normal cells that radiosensitivity itself cannot be used as a means of identifying a heterozygote in any individual case.

Striking evidence of the increased sensitivity to cancer of AT heterozygotes comes from

**TABLE 17.4.** New Cases of Cancer Occuring at Age 20 Years or Older During Follow-up of 161 Families Affected by Ataxia Telangiectasia

Primary Site	Blood Relatives (91/1,599 = 5.7%)		
	Obligate Heterozygotes	All Others	Spouses (19/821 = 2.3%)
Breast	5	18	3
All other	13	55	16
Total	18	73	19

From Swift M, Morrell D, Massey RB, Chase CL: N Engl J Med 325:1831-1836. 1991, with permission.

**TABLE 17.5.** Rate Ratios and Estimates of the Risk of Cancer Among Persons 20- to 79-Years-Old in Families Affected by Ataxia Telangiectasia

Sex and Type of Cancer	Rate Ratios		
	All Blood Relatives	Obligate Heterozygotes <sup>a</sup>	Estimated Risk in Heterozygotes
Men, all types	2.5	3.9	3.8
Women			
All types	2.1 <sup>a</sup>	2.7 <sup>a</sup>	3.5 <sup>a</sup>
Breast	3.3 <sup>a</sup>	3.8 <sup>a</sup>	5.1

Estimated risk refers to cases of cancer per 1,000 person-years.

From Swift M, Morrell D, Massey RB, Chase CL: N Engl J Med 325:1831-1836, 1991; with permission.

a review by Swift of 161 families affected by AT. In this prospective study, new cases of cancers were observed in blood relatives of persons with AT (of whom about half may be heterozygotic), in those who are definite heterozygotes (obligates), and in spouses who were assumed to be normal but who lived in the same environment. The crude results are shown in Table 17.4 and converted to rate ratios in Table 17.5. It is quite clear that heterozygotes are at an increased risk for all types of cancer, with breast cancer in women being prominent. Although persons who are heterozygotic for AT may comprise only 1 to 3% of the white population in the United States, these persons may account for a substantial proportion (9-18%) of all breast cancer in younger women.

This extensive study also divided blood relatives of AT homozygotes into those with, and those without, a "radiation history." A radiation history was interpreted loosely as fluoroscopy of the chest, back, or abdomen, therapeutic irradiation, or occupational exposure. Table 17.6 shows the results of the survey. Fifty-three percent of blood relatives with cancer had a radiation history, compared with 19% of those without cancer.

From these data the study purported to show that AT heterozygotes are very sensitive to radiation-induced cancer. A case-control study of this kind does not provide proof of this, but nevertheless the possibility exists. It is a challenging and sobering thought to the diagnostic radiologists that a proportion of the women routinely screened by mammography may be exquisitely sensitive to radiation-induced carcinogenesis because of repair deficiencies associated with being heterozygotic for AT.

Ataxia telangiectasia mutant cells show defects in the Gi/S, S, and G<sub>2</sub>/M checkpoints, indicating that the ATM gene is a common element in all three responses. It is clearly upstream of p53 in the Gi/S checkpoint.

p53 has been described as "the guardian of the genome" and clearly plays a central role. Individuals with the cancer-predisposing Li-Fraumeni syndrome are born with mutations in one allele of the p53 gene and develop tumors that have mutations in both alleles. The lack of normal p53 in naturally occurring tumors argues that p53 acts as a true tumor-suppressor gene, controlling tumor growth and cell division. p53 is required for DNA damage-dependent cell-cycle checkpoint at

**TABLE 17.6.** Breast Cancer and Radiation in 161 Families With Ataxia Telangiectasia

	Blood Relatives Relatives with Cancer	Blood Relatives without Cancer
Number With Radiation History <sup>3</sup>	19 10/19=53%	57 11/57=19%

<sup>a</sup>Radiation history includes fluoroscopy of chest, back, or abdomen; therapeutic radiation; and occupational exposure. From Swift M, Morrell D, Massey RB, Chase CL: N Engl J Med 325:1831-1836, 1991, with permission.

the Gi/S boundary and radiation-induced apoptosis.

### GENOMIC IMPRINTING

With the notable exception of the sex chromosomes, both parents contribute equally to the genetic makeup of their offspring. Although two copies of each gene are present, however, there are certain instances in which only one allele is expressed. This is termed **genomic imprinting**.

The imprinting that causes a specific gene to not be expressed results from DNA methylation or a chromatin structure change. It is parent-of-origin specific, and it is erased if passed through the opposite sex. Imprinting varies with the individual, the species, the tissue, and time. It plays a critical role in fetal development; it can be turned on and off at different times.

It is important in oncology because it can lead to the inheritance of cancer susceptibility in a *nonmendelian* fashion. The tumor-suppressor gene m6P/IGF 2R is a case in point. Rats and mice are imprinted for this gene; most humans are not. About 20 imprinted genes are known in the human, but it is suspected that there are many more. Several are implicated in carcinogenesis, including one oncogene and several tumor-suppressor genes. This leads to loss of heterozygosity and therefore to cancer susceptibility. Wilms' tumor is an example. If imprinted, it is always paternally imprinted and therefore maternally expressed.

Tumor susceptibility because of loss of heterozygosity caused by imprinting is therefore inherited in a nonmendelian fashion. For example, in the case of a paternally imprinted, maternally expressed gene (such as Wilms' tumor), males *always* would pass on an inactive gene; females always would pass on an expressed gene. This may confuse identification in regular linkage analysis.

### TRANSGENIC MICE

The ability to construct strains of mice with *in vitro* altered genes in place of inherent cor-

responding genes has allowed the creation of animal models for human disease. The first examples of these types of animals were reported in the early 1980s, when exogenous genes were microinjected into single-cell mouse embryos and randomly integrated into chromosomes. Embryos were implanted into foster mothers, that is, female mice made pseudopregnant by mating with vasectomized males. After birth, those offspring that had taken up the transgene were identified by Southern blotting of DNA from a small piece of the tail. Mice that carry the foreign gene are referred to as transgenic. In some cases, these animals were capable of passing on the newly introduced genetic material to offspring through germ-line transmission. The first animals created by this method contained the full complement of mouse genes and, in addition, the DNA introduced.

The techniques since have been refined, and now mice can be created in which inherent genes are replaced by *in vitro* altered genetic material through homologous recombination mechanisms. For this procedure, mouse embryonic stem cells, which are capable of differentiating into every cell type, act as the recipient of altered mouse DNA.

If a gene is inserted into a mammalian cell, there is no way to control where it recombines into the host genome. It is most likely to recombine into an unrelated sequence, in which case it is referred to as **heterologous recombination**. Occasionally, the gene may recombine precisely into the identical sequence in the genome by **homologous recombination**; this is a powerful tool to inactivate mammalian genes by incorporating a disrupted gene construct into the gene of interest. Because homologous recombination is rare, occurring roughly once per thousand heterologous insertions, gene targeting by this method relies on the ability to enrich a population of transfected cells **for** those with homologous recombination by one of the several available specialized selection processes, the details of which are beyond the scope of this chapter. The cells bearing the new mouse gene then can be in-

troduced into mouse blastocysts, where they divide, integrate into the normal cell population, and develop as part of the newborn animal. Mice found to have the exogenous gene homologously integrated into a chromosome and in germ-line cells can be mated, and diploid mutant animals thus can be produced.

### Gain-of-Function Mutations

Transgenic mice have been generated by the introduction of modified proto-oncogenes. Many have been produced including activated *myc* and *ras*.

### Loss-of-Function ("Knockout") Mutations

Gene inactivation by homologous recombination in embryonic cells has provided mutant mouse models mimicking many human inherited diseases. Mice lacking one or both alleles of known tumor-suppressor genes have been generated to evaluate the normal function of these genes *in vivo*. As expected, these mice have proved to be highly susceptible to tumor development. Knockout mouse models also have been made with germline-inactivated genes that have a general role in cellular defense against mutation and carcinogenesis, such as the cell-cycle check-point genes p53 and ATM.

### SUMMARY OF PERTINENT CONCLUSIONS

- Cancer is thought to be a clonal disorder.
- The control of cell proliferation is the consequence of signals that may be positive or negative.
- Gain-of-function mutations can activate oncogenes, which are positive growth regulators; loss of function of a tumor-suppressor gene product is a negative growth regulator.
- Oncogenes are genes that are found in either a mutant or abnormally expressed form in many human cancers.
- Oncogenes can be activated by a point mutation, a chromosomal rearrangement such as a translocation, or gene amplification.
- Some human leukemias and lymphomas appear to be caused by specific chromosomal translocations that lead to oncogene activation in several different ways.
- The concept of tumor-suppressor genes arose from the *in vitro* observation that the fusion of a normal human fibroblast with a HeLa cell suppressed the expression of the malignant phenotype of the HeLa cell.
- Knudsen postulated that all types of retinoblastoma involve two separate mutations. In sporadic retinoblastoma both mutations occur somatically in the same retinal cell during *in utero* life—therefore this condition is rare. In the heritable form, one of the two mutations is inherited from a parent and is present in all retinal cells, so that the second mutation would occur somatically in any of these cells—hence the incidence is close to 100%.
- There are many tumor-suppressor genes whose location and function are known; the two most intensively studied are p53 and Rb.
- Because oncogenes are gain-of-function mutations, only one copy needs to be activated; that is, they act in a dominant fashion. Tumor-suppressor genes involve loss-of-function mutations, so that both copies must be lost; that is, they act in a recessive fashion.
- Somatic homozygosity is the process by which one chromosome of a pair is lost, a deletion occurs in the remaining chromosome, and the chromosome with the deletion replicates.

- Carcinogenesis appears to be a multistep process with multiple genetic alterations occurring. An attractive model of carcinogenesis includes the idea that an early step causes a mutation in a gene in one of the families responsible for the stability of the genome. This leads to a mutator phenotype, so that multiple further changes are likely in the progression of the cancer.
- Telomeres cap the ends of chromosomes; they are long arrays of TTAGGG repeats. Each time a normal somatic cell divides, the terminal end of the telomere is lost; after 40 to 60 divisions, the cell undergoes senescence. Stem cells and cancer cells activate telomerase, which maintains telomere length so that the cell becomes immortal.
- Signal transduction is the term used to describe the flow of information from the outer membrane of the cell into the nucleus. Multiple signal-transduction pathways can operate simultaneously in tandem. One pathway includes the GTP-binding proteins, including the *ras* family; this is important because *ras* frequently is mutated in human cancers. Another example is the protein kinase C signal transduction pathway.
- Low doses of ionizing radiation can up-regulate or down-regulate a host of early-responding genes.
- The transcriptional activity of p53 is modulated in response to DNA damage by the activity of a number of kinases, as well as by protein-protein interactions resulting in either cell-cycle arrest or apoptosis.
- Ionizing radiations can activate both nuclear and membrane-cytoplasmic signal transduction pathways. This can lead to, for example, cell-cycle arrest or, alternatively, apoptosis.
- The division of the cell cycle into its constituent phases, M, Gi, S, and G2 was accomplished in the 1950s.
- The arrest of cells at various positions in the cycle by the action of "checkpoint genes" is an important response to DNA damage. The two principle checkpoints are the Gi/S and the G2/M boundary, but there is also a checkpoint in S. G2/M is the most well-known checkpoint following radiation damage; cells pause at G2/M to repair damage before attempting the complex process of mitosis.
- Progression through the cell cycle is governed by protein kinases, activated by cyclins. Each cyclin protein is synthesized at a discrete phase of the cycle: cyclin D and E in Gi, cyclin A in S and G2, and cyclin B in G2 and M. Transitions in the cycle occur only if a given kinase activates the proteins required for progression.
- There are a number of hereditary disorders that predispose to cancer. There are several ways that this can occur:
  1. A germline mutation in a tumor-suppressor gene
  2. Germline activation of an oncogene
  3. Any syndrome that includes DNA repair defects
  4. Unusual sensitivity to carcinogens because of defects in metabolism.
- *Genomic imprinting* is the term used if only one allele is expressed, although both copies of the gene are present. It can lead to inheritance of cancer susceptibility in a non-mendelian fashion.
- Transgenic mice are animals with *in vitro* altered genes in place of inherent corresponding genes. This allows the creation of animal models for human disease. Knockout mice have been produced, lacking one or both alleles of known tumor-suppressor genes as well as genes that have a more general role, such as p53 and ATM.

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# 18

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## Dose-Response Relationships for Model Normal Tissues

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Mitotic Death and Apoptosis: How and Why Cells Die  
Assays for Dose-Response Relationships  
Clonogenic Endpoints  
Summary of Dose-Response Curves  
for Clonogenic Assays in Normal Tissues

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Dose-Response Relationships for Functional Endpoints  
Inferring the Ratio  $a/3$  from Multifraction Experiments in Nonclonogenic Systems  
Summary of Pertinent Conclusions

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### MITOTIC DEATH AND APOPTOSIS: HOW AND WHY CELLS DIE

Most cell lines cultured *in vitro* die a mitotic death after irradiation; that is, they die attempting to divide. This does not necessarily occur at the first postirradiation mitosis; the cell may struggle through one, two, or more mitoses before the damaged chromosomes cause it to die attempting the complex task of cell division. Time-lapse films of irradiated cells cultured *in vitro* clearly show this process of mitotic death, which is the dominant cause of death if reproductive integrity is assessed *in vitro* as described in Chapter 3.

It is not, however, the only form of cell death. Programmed cell death, or **apoptosis**, occurs in normal tissues and neoplasms, in mammals and amphibians, in the embryo and the adult. It is implicated, for example, in tissue involution, such as the regression of the tadpole tail during metamorphosis. It is the programmed cell death that is common

during embryonic development. It also can occur after irradiation. *Apoptosis*, like *mitosis*, comes from the Greek meaning "falling off," as of petals from flowers or leaves from trees.

Apoptosis is characterized by a stereotyped sequence of morphologic events, which take place in two discrete phases. In the first phase, cells condense and bud to produce many membrane-enclosed bodies. In the second phase, these bodies are phagocytized and digested by nearby tissue cells. The characteristic "laddering" of DNA that occurs during apoptotic death is illustrated in Chapter 3. Apoptosis characteristically affects scattered individual cells. If apoptosis affects cells in tissues, the resulting apoptotic bodies are squeezed along the intercellular spaces and are either shed from the epithelial surface or rapidly phagocytized by nearby cells. The cells surrounding those being deleted merely close ranks, and there is no tissue disorganization such as occurs after necrosis.

## ASSAYS FOR DOSE-RESPONSE RELATIONSHIPS

A number of experimental techniques are available to obtain dose-response relationships for the cells of normal tissues. First, there are a limited number of clonogenic assays—techniques in which the endpoint observed depends directly on the reproductive integrity of individual cells. These systems are directly analogous to cell survival *in vitro*. The techniques developed by Withers and his colleagues are based on the observation of a clone of cells regenerating *in situ* in irradiated tissue. The skin colony, testes, kidney tubule, and regenerating crypts in the jejunum systems are described briefly later in this chapter. It is also possible to obtain dose-response curves for the cells of the epithelial lining of the colon or stomach, but the method used is essentially the same as for the jejunum. Kember described a system for scoring regenerating clones in cartilage at about the same time as the Withers's skin colony system, but it has not been used widely and is not discussed here.

The assay system for the stem cells in the bone marrow or cells of the thyroid and mammary gland depends on the observation of the growth of clones of cells taken from a donor animal and transplanted into a different tissue in a recipient animal. In Till and McCulloch's bone-marrow assay, colonies of bone marrow cells are counted in the spleens of recipient animals. Dose-response curves for mammary and thyroid cells have been obtained by Gould and Clifton by observing colonies growing from cells transplanted into the fat pad of recipient animals.

Second, dose-response relationships can be obtained that are repeatable and quantitative but that depend on functional endpoints. These include skin reactions in rodents or pigs (*e.g.*, erythema and desquamation), pneumonitis or fibrosis in mouse lungs reflected in an increased breathing rate, myelopathy of the hind limbs from damage to the spinal cord, and deformities to the feet of mice. The endpoints observed tend to reflect the minimum number of functional cells **remaining** in a tissue or or-

gan, rather than the fraction of cells retaining their reproductive integrity.

Finally, one can *infer* a dose-response curve for a tissue in which it cannot be observed directly by assuming the form of the dose-response curve (linear-quadratic) and performing a series of multifraction experiments. This procedure, first suggested by Douglas and Fowler, has been used widely to infer values for *a* and *b* in the dose-response relationships for normal tissues in which the parameters cannot be measured directly.

This chapter includes assays for both early- and late-responding tissues. The skin, intestinal epithelium, and bone-marrow cells, for example, are rapidly dividing self-renewal tissues. The spinal cord, lung, and kidney, by contrast, are late-responding tissues. This reflects the current philosophy that the radiation response of *all* tissues results from the depletion of the critical parenchymal cells, and that the difference in time at which early- and late-responding tissues express radiation damage is a function simply of different cell-turnover rates. Many older papers in the literature ascribe the response of late-responding tissues to vascular damage, rather than to depletion of parenchymal cells, but this thesis is becoming increasingly difficult to accept.

The various types of normal tissue assay systems are described briefly. The reader who is content with the summary already given may wish to skip the remainder of this chapter.

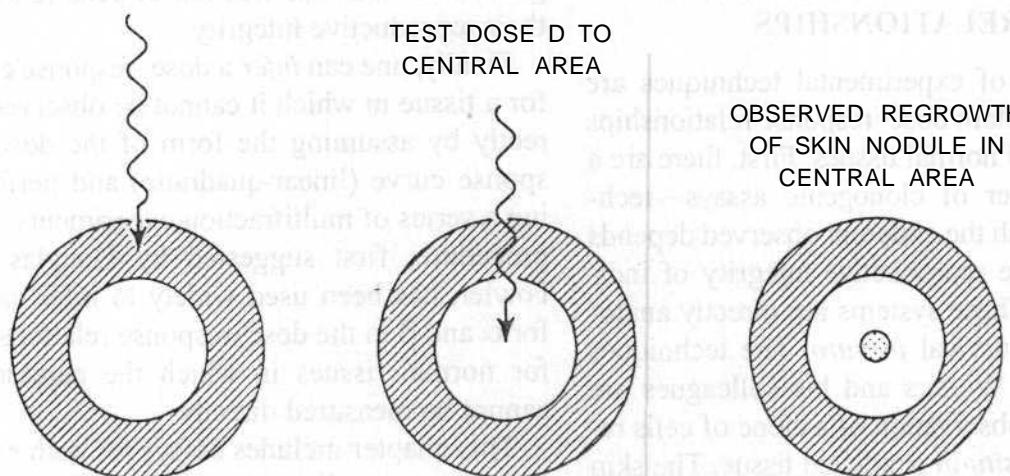
## CLONOGENIC ENDPOINTS

### Clones Regrowing // *In Situ*

#### *Skin Colonies*

Withers developed an ingenious technique (Fig. 18.1) to determine the survival curve for mouse skin cells. The hair was plucked from an area on the back of the mouse, and a superficial x-ray machine was used to irradiate an annulus of skin to a massive dose of 30 Gy (3,000 rad). This produced a "**moat**" of dead cells, in the center of which was an isolated island of intact skin that had been protected

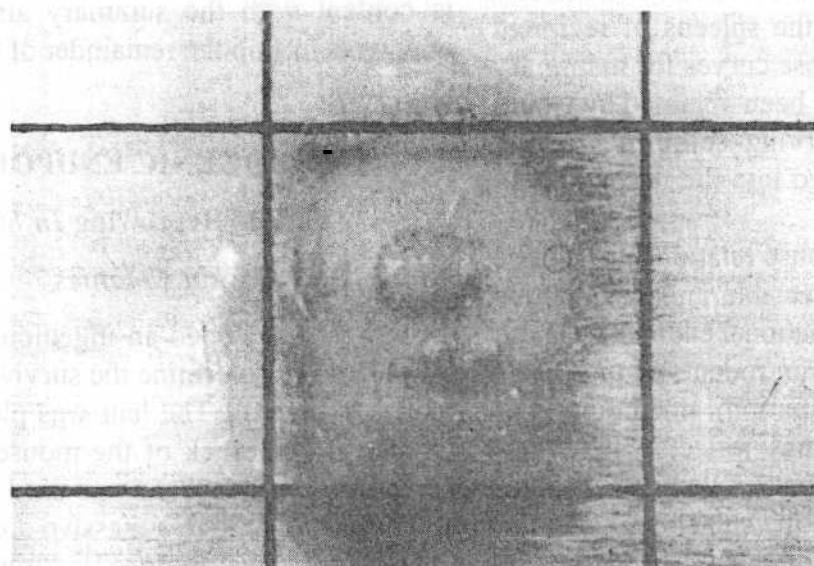
## 3000 RADS TO MOAT



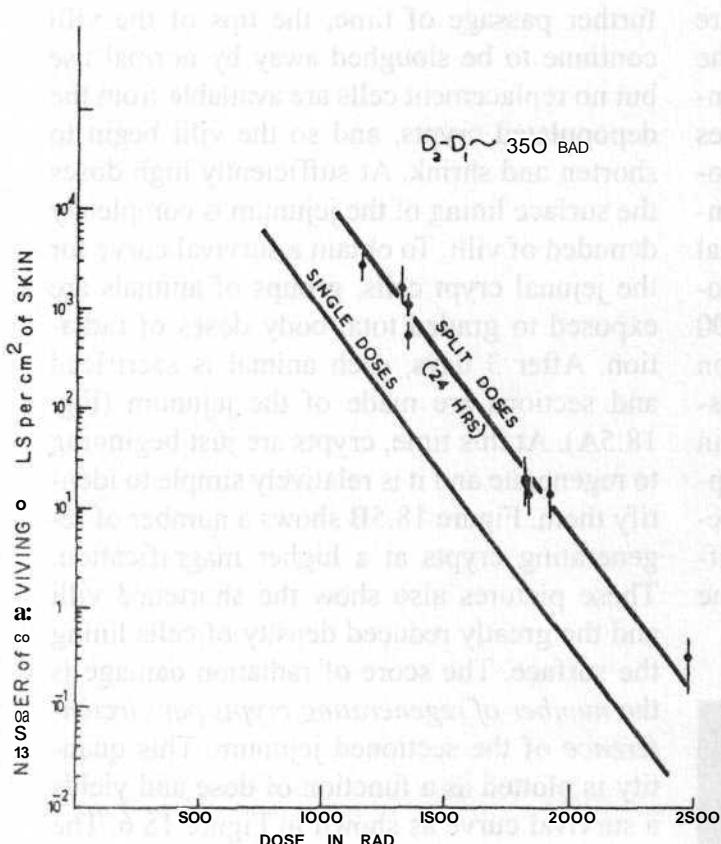
**Figure 18.1.** Technique used to isolate an area of skin for experimental irradiation. A superficial (30-kV) x-ray machine is used to irradiate an annulus of skin to a massive dose of about 3,000 rad (30 Gy). An isolated island of intact skin in the center of this "moat" is protected from the radiation by a metal sphere. The intact skin then is given a test dose (D) and observed for nodules of regrowing skin. (Adapted from Withers HR: Br J Radiol 40:187, 1967, with permission.)

during the first exposure to low-voltage x-rays by a small metal sphere. This small area of intact skin then was given a test dose (D) and subsequently observed for regrowth of skin. If one or more stem cells survived in this small area, nodules of regrowing skin could be seen some days later. If no cells survived in this small area, the skin would heal much later by infiltration of cells crossing the moat. Fig-

ure 18.2 shows nodules regrowing in mouse skin. To obtain a survival curve, it was necessary to repeat this operation with a number of different areas of skin. A range of ball bearings was used to shield a small area of skin in the middle of the "moat." The resulting survival data are shown in Figure 18.3, in which the dose is plotted against the number of surviving cells per square centimeter of skin.



**Figure 18.2.** Photograph of a nodule of mouse skin regrowing from a single surviving cell in the treated area. (Courtesy of Dr. H. R. Withers)



**Figure 18.3.** Single-dose and two-dose survival curves for epithelial cells of mouse skin exposed to 29-kVp x-rays. The 37% dose slope ( $D_0$ ) is 1.35 Gy (135 rad). The ordinate is not the surviving fraction, as in the survival curves for cells cultured *in vitro*, but is the number of surviving cells per square centimeter of skin. In the two-dose survival curve the interval between dose fractions was always 24 hours. The curves are parallel, their horizontal separation being equal to 3.5 Gy (350 rad); this corresponds to  $D_q$ . From a knowledge of  $D_q$  and the slope of the survival curve,  $D_0$ , the extrapolation number,  $n$ , may be calculated. (From Withers HR: Radiat Res 32:227, 1967; and Withers HR: Br J Radiol 40:187, 1967, with permission.)

There are practical limits to the range in which the dose-response relationship could be determined. At one extreme, it is not possible to irradiate too large an area on the back of the mouse to produce the most of sterilized skin. At the other extreme, the smallest area that can be used is determined by the fact that even 30-kV radiation scatters laterally to some extent. As can be seen in Figure 18.3, the technique results in a single-dose survival curve that extends from 8 to 25 Gy (800-2,500 rad); over this range, with dose plotted on a linear scale and the number of surviving cells per square centimeter plotted on a logarithmic scale, the survival curve is straight and has a  $D_0$  of 1.35 Gy (135 rad). This  $D_0$  value is very similar to that obtained with mammalian cells cultured *in vitro*.

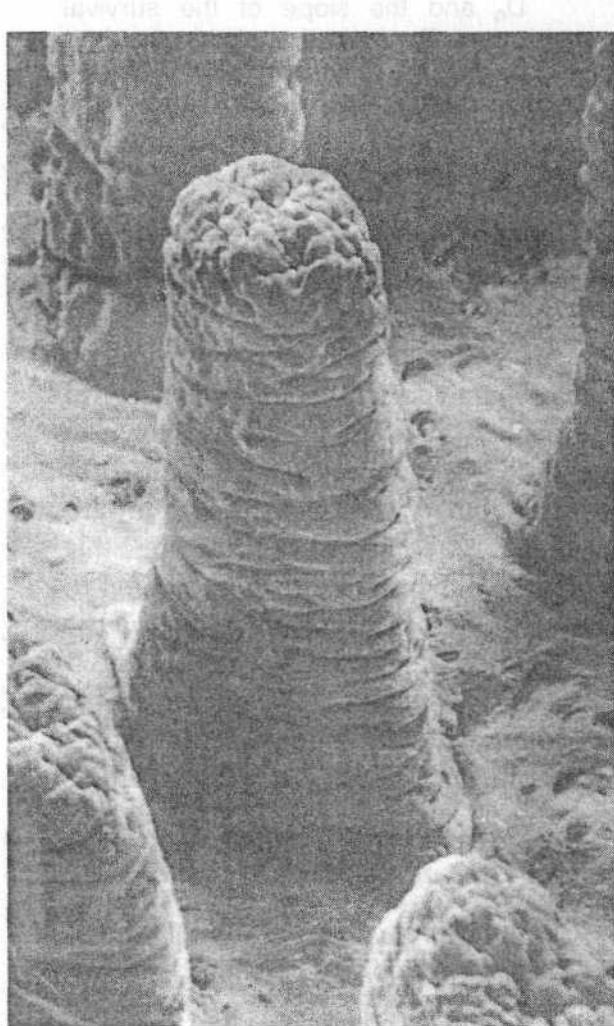
The extrapolation number cannot be obtained directly with this technique, because the ordinate is the number of surviving cells per square centimeter of skin; this cannot be converted to the surviving fraction because it is not known with any accuracy how many skin stem cells there are per unit area. It is,

however, possible to make an indirect estimate of the extrapolation number by obtaining the survival curve for doses given in two fractions separated by 24 hours. The survival curve obtained in this way also is shown in Figure 18.3. It is parallel to that obtained for single doses but is displaced from it toward higher doses. As explained in Chapter 3, this lateral displacement in a direction parallel to the dose axis is a measure of  $D_q$ , *the quasi-threshold dose*. The  $D_q$  for mouse skin is 3.5 Gy (350 rad), which is very similar to the value for human skin estimated from split-dose experiments.

#### Crypt Cells of the Mouse Jejunum

A technique perfected by Withers and Elkind makes it possible to obtain the survival characteristics of the crypt cells of the mouse jejunum. The lining of the jejunum is a classic example of a self-renewal system. The cells in the crypts divide rapidly and provide a continuous supply of cells that move up the villi, differentiate, and become the functioning cells.

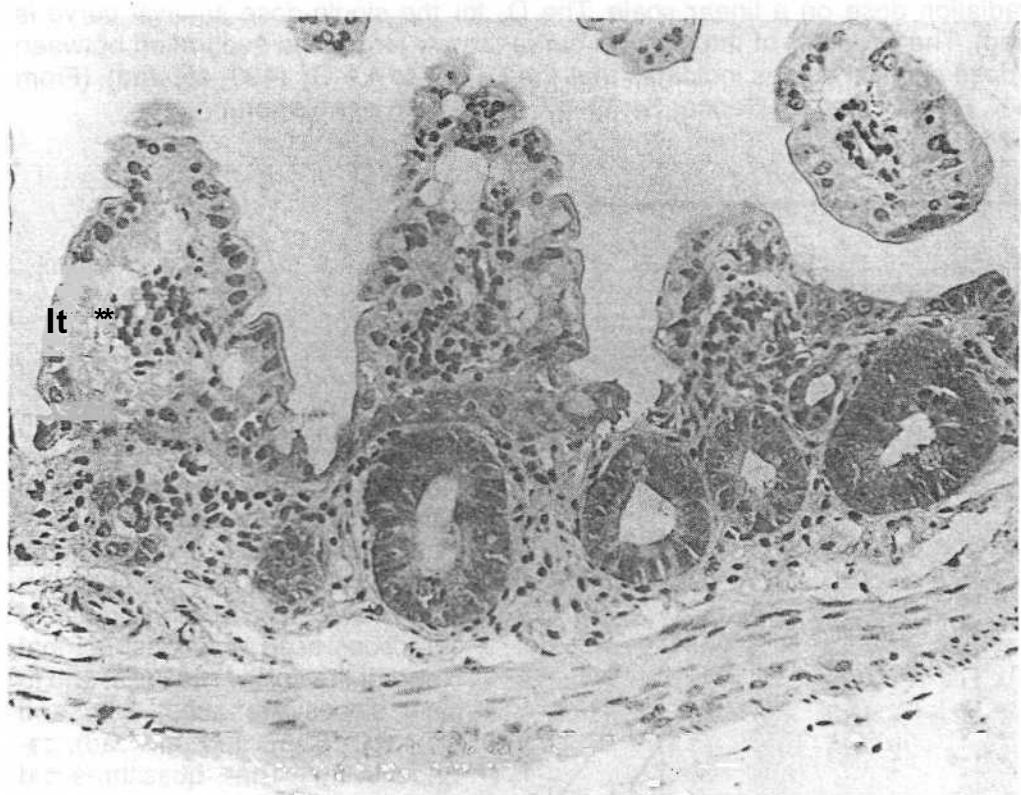
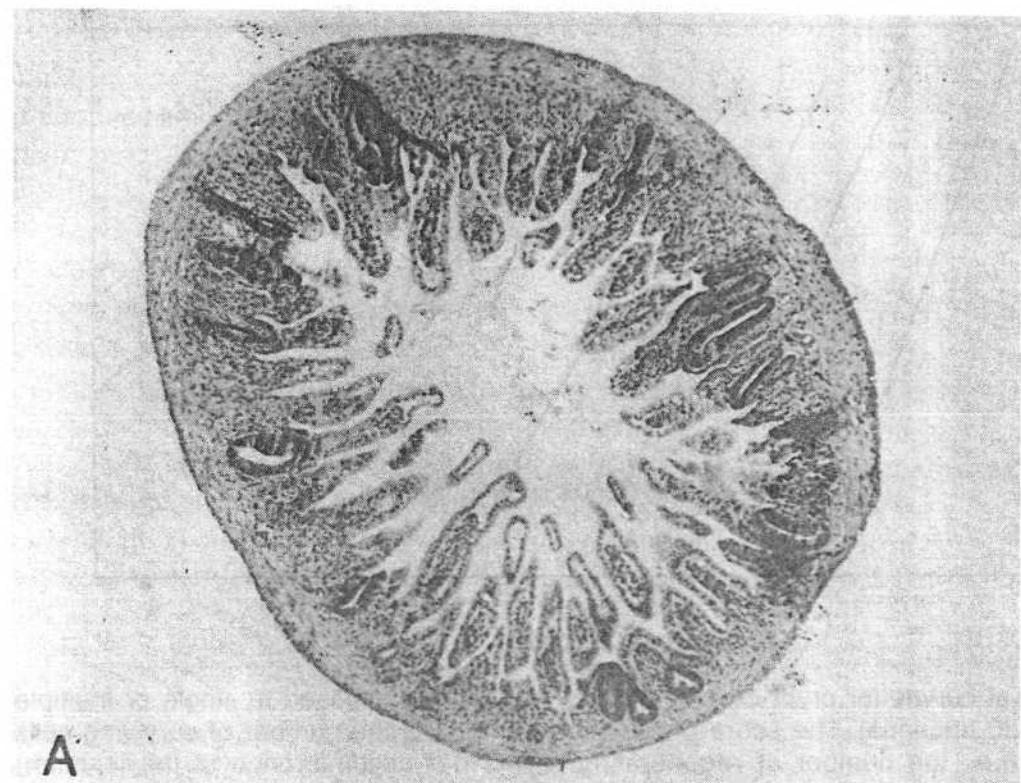
The cells at the top of the folds of the villi are slowly but continuously sloughed off in the normal course of events and are replaced continuously by cells that originate from mitoses in the crypts. Figure 18.4, an electron micrograph, dramatically shows the three-dimensional structure of the lining of the intestinal epithelium in the mouse. Mice are given a total-body dose of 11 to 16 Gy (1,100-1,600 rad), which sterilizes a significant proportion of the dividing cells in the crypts but has essentially no effect on the differentiated cells in the villi. Consequently, crypt degeneration appears early after irradiation, and the villi remain long and their epithelial covering of differentiated cells shows little change. With the



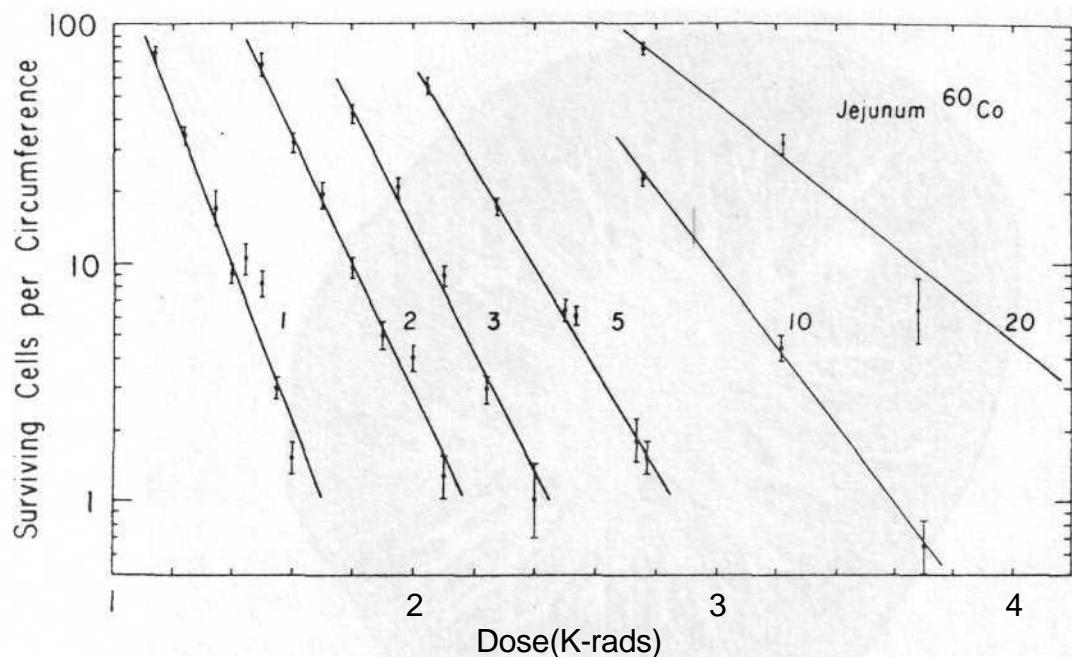
**Figure 18.4.** Scanning electron micrograph that allows three-dimensional visualization of the jejunal villi from the hamster. (Magnification  $\times 175$ .) (From Taylor AB, Anderson JH: *Micron* 3:430-453, 1972, with permission.)

further passage of time, the tips of the villi continue to be sloughed away by normal use but no replacement cells are available from the depopulated crypts, and so the villi begin to shorten and shrink. At sufficiently high doses the surface lining of the jejunum is completely denuded of villi. To obtain a survival curve for the jejunal crypt cells, groups of animals are exposed to graded total-body doses of radiation. After 3 days, each animal is sacrificed and sections are made of the jejunum (Fig. 18.5A). At this time, crypts are just beginning to regenerate and it is relatively simple to identify them. Figure 18.5B shows a number of regenerating crypts at a higher magnification. These pictures also show the shortened villi and the greatly reduced density of cells lining the surface. The score of radiation damage is the *number of regenerating crypts per circumference* of the sectioned jejunum. This quantity is plotted as a function of dose and yields a survival curve as shown in Figure 18.6. The single-dose survival curve has a  $D_0$  (for  $\gamma$ -rays) of about 1.3 Gy (130 rad). Also shown in Figure 18.6 are survival curves for radiation delivered in multiple fractions, from 2 to 20. The separation between the single- and two-dose survival curves gives a measure of  $D_q$ , which has the very large value of between 4 and 4.5 Gy (400 $\times$ 50 rad).

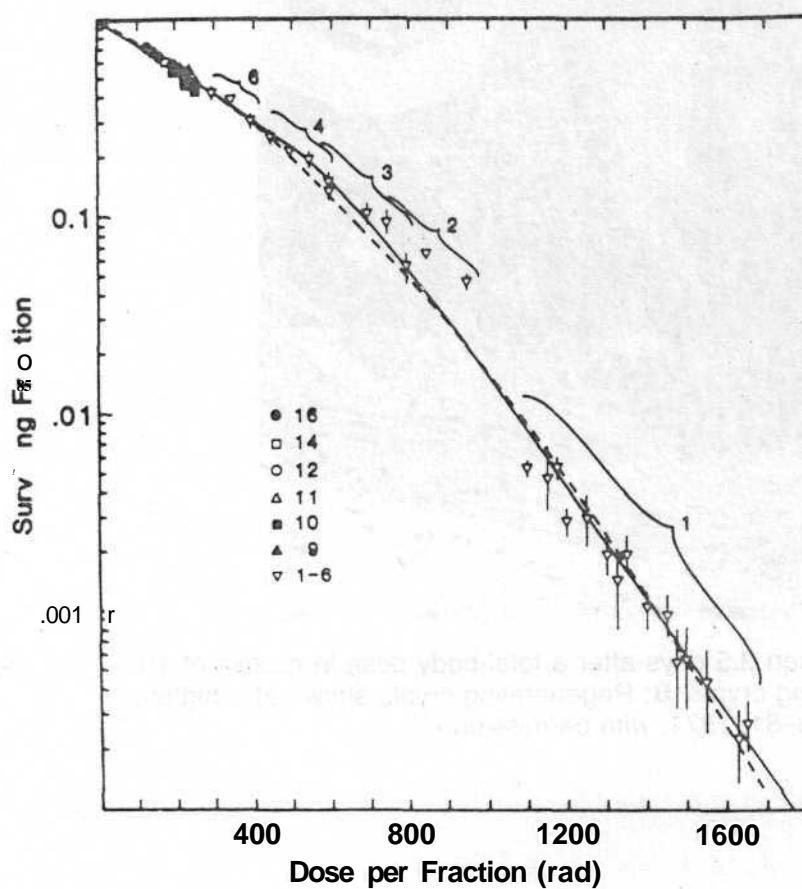
This technique has two limitations. First, the quantity plotted on the ordinate is the number of surviving crypts per circumference, not the surviving fraction. Second, experiments can be done only at doses of about 10 Gy (1,000 rad) or more, at which there is a sufficient level of biologic damage for individual regenerating crypts to be identified. The doses can be delivered, however, in a number of smaller fractions, as long as the total results in enough biologic damage to be scored. The shape of the entire survival curve then can be reconstructed from the multifraction data, if it is assumed that in a fractionated regimen each dose produces the same amount of cell killing, and if an estimate is made of the number of clonogens at risk per crypt. This has been done by Withers and his colleagues; the resultant survival curve is shown in Figure 18.7.



**Figure 18.5. A:** Section of mouse jejunum taken 3.5 days after a total-body dose in excess of 10 Gy. Note the shortened villi and the regenerating crypts. **B:** Regenerating crypts shown at a higher magnification. (From Withers HR: Cancer 28:78-81, 1971, with permission.)



**Figure 18.6.** Survival curves for crypt cells in the mouse jejunum exposed to single or multiple doses of  $\gamma$ -rays (1-20 fractions). The score of radiation damage is the number of surviving cells per circumference (i.e., the number of regenerating crypts per circumference of the jejunum) counted from sections such as those shown in Figure 18.5. This quantity is plotted on a logarithmic scale against radiation dose on a linear scale. The  $D_0$  for the single-dose survival curve is about 1.3 Gy (130 rad). The shoulder of the survival curve is very large. The separation between the single- and two-dose survival curves indicates that the  $D_q$  is 4 to 4.5 Gy (400-450 rad). (From Withers HR, Mason K, Reid BO, et al.: Cancer 34:39-47, 1974, with permission.)



**Figure 18.7.** Effective single-dose survival curve reconstructed from multifraction experiments for clonogenic cells of the jejunal crypts of mice. The numbers on the curve refer to the number of fractions used to reconstruct that part of the curve. The initial and final slopes are about 3.57 and 1.43 Gy (357 and 143 rad), respectively. The quasithreshold dose is 4.3 Gy (430 rad). The data are equally well fitted by the linear-quadratic formulation. (From Thames HD, Withers R, Mason KA, Reid BO: Dose survival characteristics of mouse jejunal crypt cells. Int J Radiat Oncol Biol Phys 7:1591-1597, 1981, with permission.)

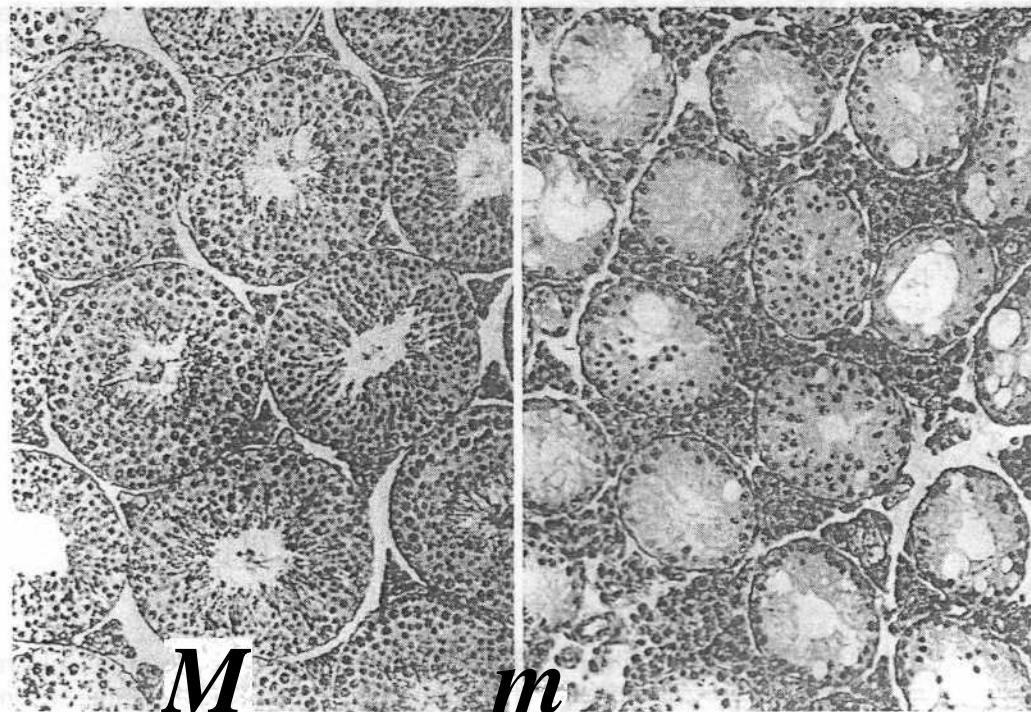
### Tastes Stem Cells

A technique to measure the radiation response of testicular cells capable of sustaining spermatogenesis (*i.e.*, the stem cells) was devised by Withers and his colleagues. About 6 weeks after irradiation, mouse testes are sectioned and examined histologically. Sections of normal and irradiated testes are shown in Figure 18.8. The proportion of tubules containing spermatogenic epithelium is counted and plotted as a function of dose in Figure 18.9. As in many *in vivo* assays, relatively high single doses of 8 to 16 Gy (800-1,600 rad) are necessary, so that the level of damage is sufficient to be scored. In this dose range,  $D_0$  is about 1.68 Gy (168 rad). If the split-dose technique is used, the  $D_q$  is about 2.7 Gy (270 rad) (Fig. 18.9). It is possible to estimate the effect of small doses and reconstruct a complete sur-

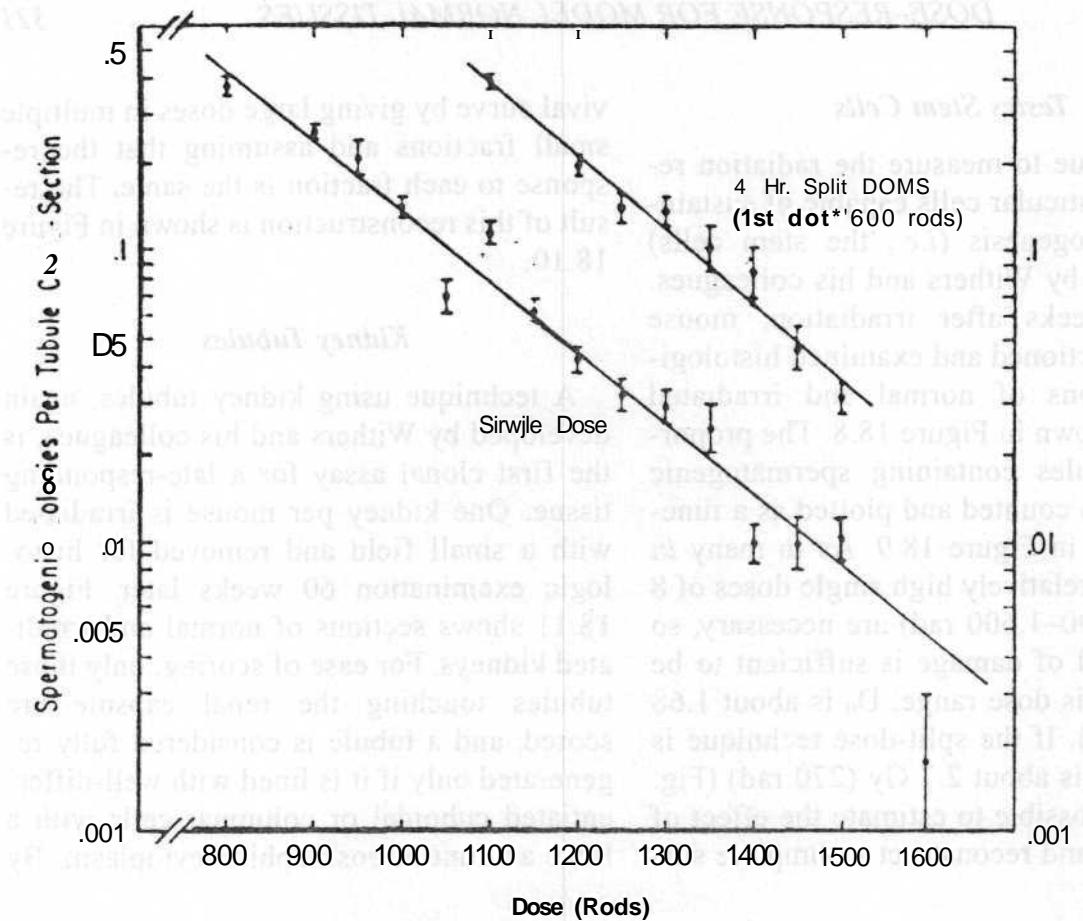
vival curve by giving large doses in multiple small fractions and assuming that the response to each fraction is the same. The result of this reconstruction is shown in Figure 18.10.

### Kidney Tubules

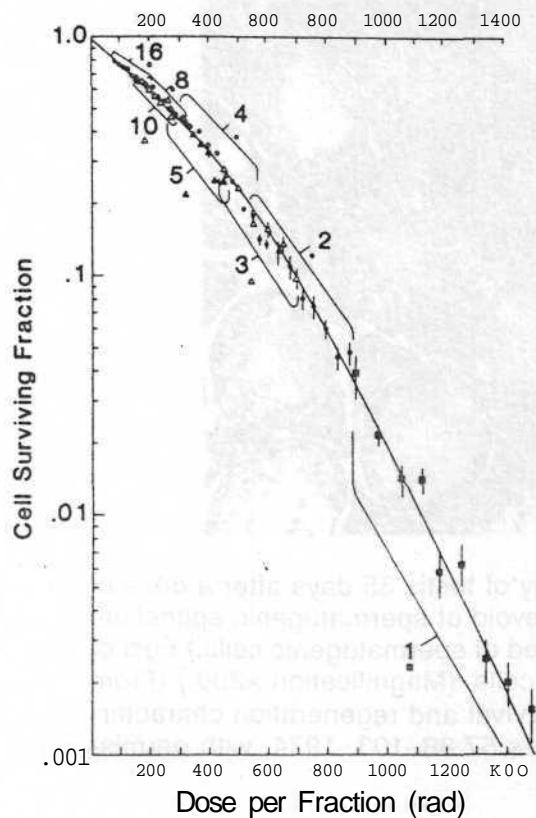
A technique using kidney tubules, again developed by Withers and his colleagues, is the first clonal assay for a late-responding tissue. One kidney per mouse is irradiated with a small field and removed for histologic examination 60 weeks later. Figure 18.11 shows sections of normal and irradiated kidneys. For ease of scoring, only those tubules touching the renal capsule are scored, and a tubule is considered fully regenerated only if it is lined **with** well-differentiated cuboidal or columnar cells **with** a large amount of eosinophilic cytoplasm. By



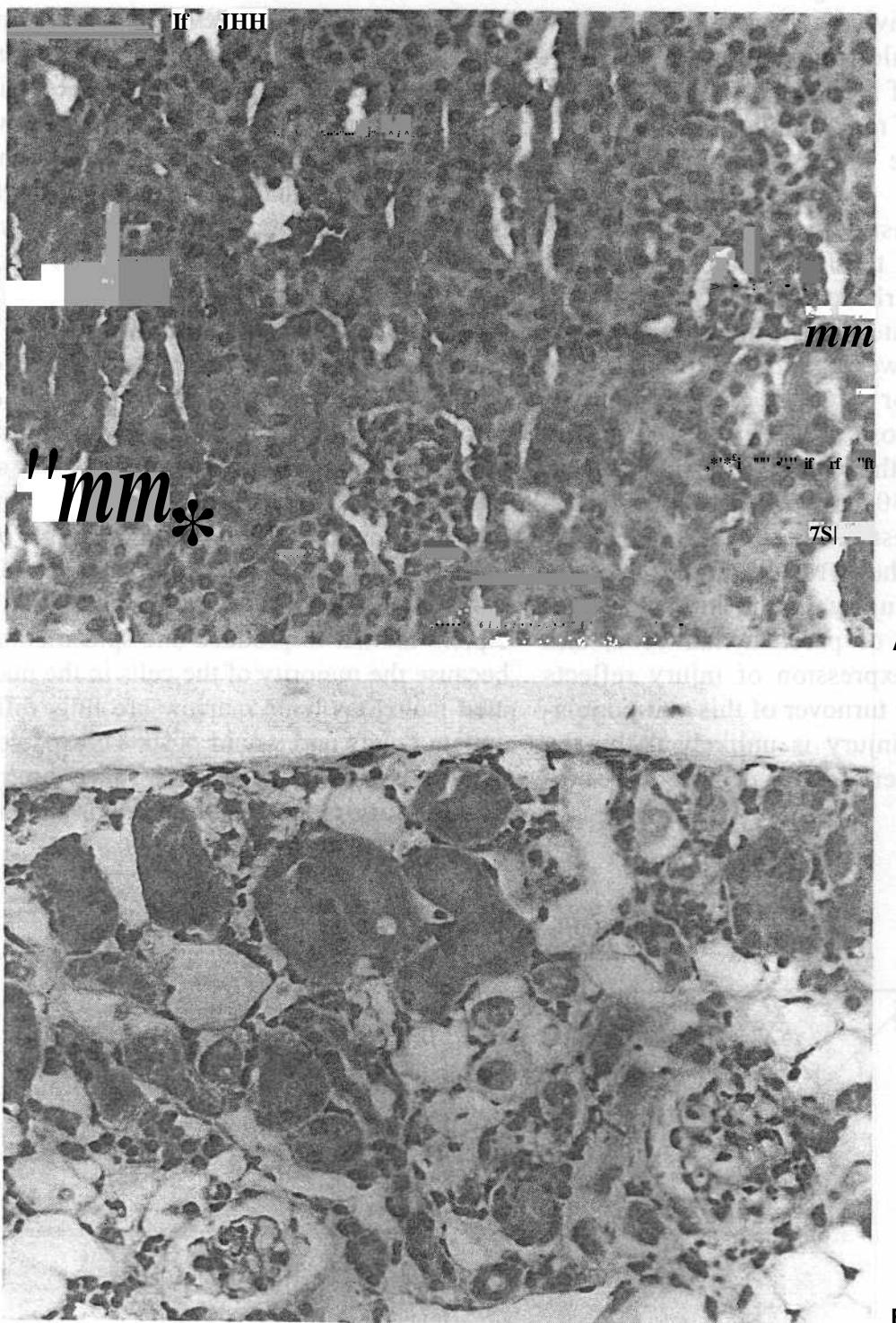
**Figure 18.8. Left:** Histology of normal testis. **Right:** Histology of testis 35 days after a dose of 9 Gy (900 rad) of  $\gamma$ -radiation. Some tubules are completely devoid of spermatogenic epithelium and some are not. (Sertoli's cells persist in the tubules sterilized of spermatogenic cells.) Foci of spermatogenesis can be derived from single surviving stem cells. (Magnification x200.) (From Withers HR, Hunter N, Barkley HT Jr, Reid BO: Radiation survival and regeneration characteristics of spermatogenic stem cells of mouse testis. Radiat Res 57:88-103, 1974, with permission.)



**Figure 18.9.** Single- and split-dose survival curves for spermatogenic stem cells of the mouse testis. The Do is about 1.68 Gy (168 rad). The  $D_q$ , assessed from the horizontal separation of the single- and split-dose curves, is about 2.7 Gy (270 rad). (From Withers HR, Hunter N, Barkley HT Jr, Reid BO: Radiation survival and regeneration characteristics of spermatogenic stem cells of mouse testis. Radiat Res 57:88-103, 1974, with permission.)



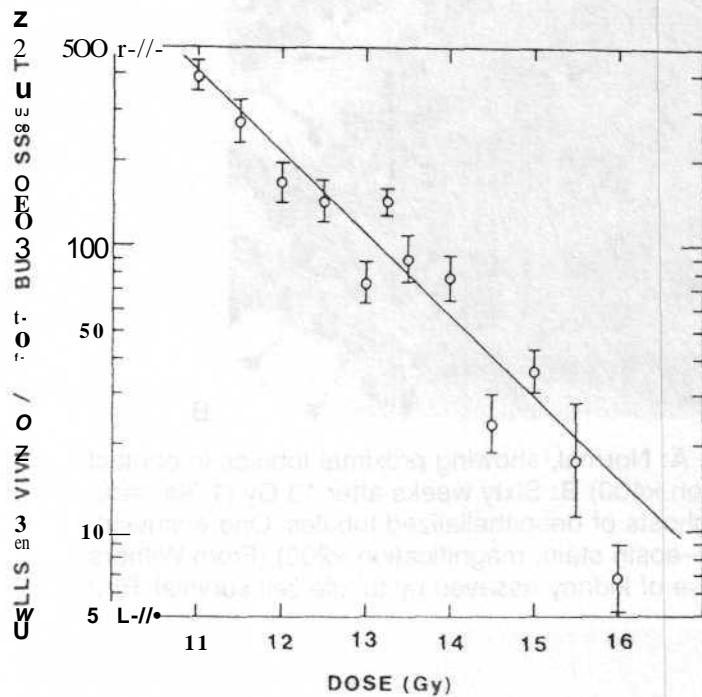
**Figure 18.10.** Survival curve for testis stem cells reconstructed from multifraction experiments, assuming that each fraction produces the same biologic effect. The numbers on the curve refer to the number of fractions used to reconstruct that portion of the curve. The Do is about 1.6 Gy (160 rad), and the  $D_q$  is about 3.92 Gy (392 rad). (From Thames HD, Withers HR: Test of equal effect per fraction and estimation of initial clonogen number in microcolony assays of survival after fractionated irradiation. Br J Radiol 53:1071-1077, 1980, with permission.)



**Figure 18.11.** Photomicrographs of mouse kidney. A: Normal, showing proximal tubules in contact with capsule. (Hematoxylin-eosin stain, magnification x400) B: Sixty weeks after 13 Gy (1,300 rad). Note normal proximal tubules and glomeruli amid ghosts of deepithelialized tubules. One epithelialized tubule is in contact with capsule. (Hematoxylin-eosin stain, magnification x200) (From Withers HR, Mason KA, Thames HD: Late radiation response of kidney assayed by tubule cell survival. Br J Radiol 59:587-595, 1986, with permission.)

60 weeks, tubules either have no surviving epithelial cells or are lined completely with epithelium that has regenerated from a small number of surviving cells, usually one. The number of tubules regenerating in an arbitrary number of sections counted is plotted as a function of radiation dose. The result is shown in Figure 18.12;  $D_0$  is about 1.53 Gy (153 rad).

The radiosensitivity of the cells of this late-responding tissue is not very different from that of early-responding tissues, such as the skin or intestinal epithelium. The *rate* of response, however, is quite different. The time required for depletion of the epithelium after a single dose of 14 Gy (1,400 rad) is about 3 days in the jejunum, 12 to 24 days in the skin, and 30 days in the seminiferous tubules of the testes, but 300 days in the kidney tubules. These results argue strongly that radiation injury in the kidney results from depletion of parenchymal cells, and that the slow expression of injury reflects merely the slow turnover of this cell population. Vascular injury is unlikely to be the mechanism underlying the destruction of renal tubules.



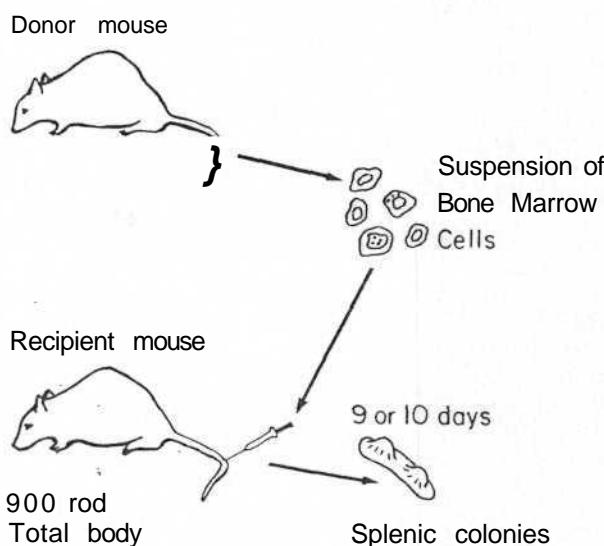
### Cells Transplanted to Another Site

#### Bone-marrow Stem Cells

Till and McCulloch developed a system to determine a survival curve for colony-forming bone-marrow cells (Fig. 18.13). Recipient animals first are irradiated supralethally with a dose of 9 to 10 Gy (900-1,000 rad), which sterilizes their spleens. Nucleated isologous bone-marrow cells taken from another animal then are injected intravenously into the recipient animals. Some of these cells lodge in the spleen, in which they form nodules, or colonies, 10 to 11 days later, because the cells of the recipient animal's spleen have been sterilized previously by the large dose of radiation. At about 10 days, therefore, the animals' spleens are removed and the colonies counted. Figure 18.14 is a photograph of a spleen showing the colonies to be counted.

About  $10^4$  cells must be injected into a recipient animal to produce one spleen colony, because the majority of the cells in the nucleated isologous bone marrow are fully differentiated cells and would never be capable of forming a colony. To obtain a surviving fraction for bone-marrow cells, a donor animal is

**Figure 18.12.** Dose-survival curve for tubule-regenerating cells. The  $D_0$  is 1.53 Gy (153 rad). (From Withers HR, Mason KA, Thames HD Jr: Br J Radiol 59:587-595, 1986)



**Figure 18.13.** Till and McCulloch's technique. From the donor mouse a cell suspension is made of nucleated isologous bone marrow. A known number of cells is injected into recipient mice previously irradiated with 9 Gy (900 rad) total-body dose. The spleen is removed from each recipient mouse 9 or 10 days later, and the number of nodules are counted. (Adapted from Till JE, McCulloch EA: In Cameron IL, Padilla GM, Zimmerman AM [eds]: Developmental Aspects of the Cell Cycle, pp 297-313. New York, Academic Press, 1971, with permission.)

irradiated to some test dose, and the suspension of cells from the bone marrow is inoculated into groups of recipient animals that previously had been irradiated supralethally. By counting the colonies in the spleens of the recipient animals, and with a knowledge of the

number of cells required to produce a colony in an unirradiated animal (plating efficiency), the surviving fraction may be calculated simply as follows:

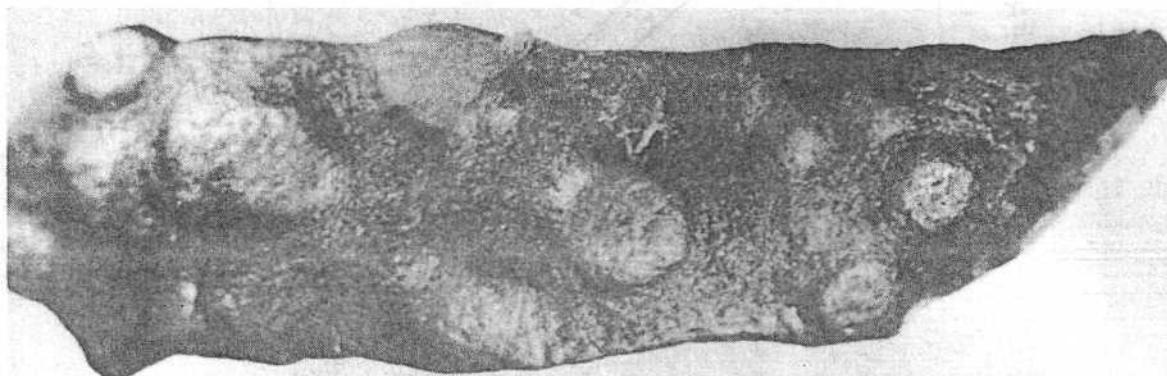
$$\text{Surviving fraction for a dose D} = \frac{\text{Colonies counted}}{\text{Cells inoculated}} \times \text{PE}$$

This procedure is repeated for a range of doses, and a survival curve is obtained (Fig. 18.15). These bone-marrow stem cells are the most sensitive mammalian cells to die a mitotic death, with a Do of about 0.95 Gy (95 rad) and little or no shoulder to the survival curve.

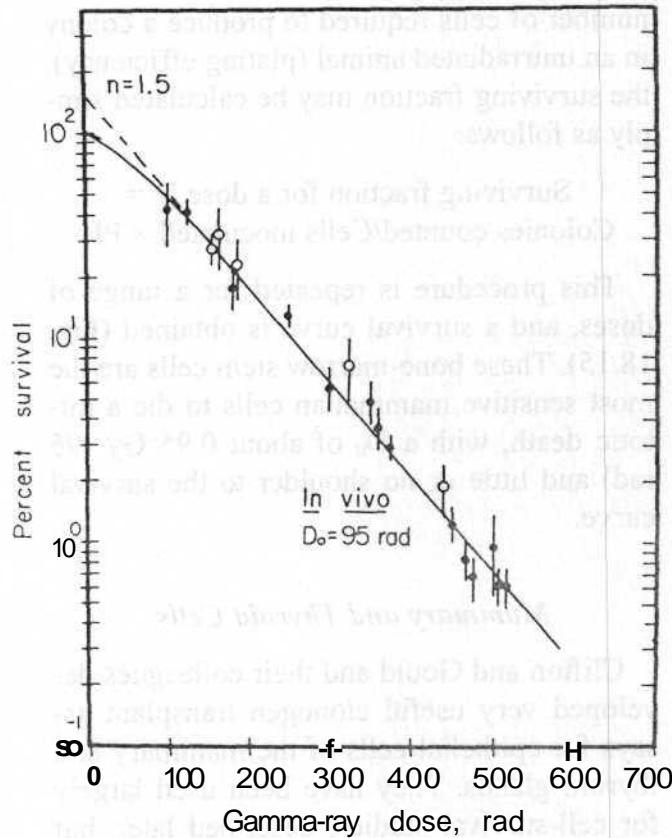
### Mammary and Thyroid Cells

Clifton and Gould and their colleagues developed very useful clonogen transplant assays for epithelial cells of the mammary and thyroid glands. They have been used largely for cell-survival studies, described later, but the initial motivation for their development was to study carcinogenesis in a quantitative system. Most *in vitro* transformation assays involve fibroblasts, and the bulk of human cancers arise in epithelial cells; hence, the importance and interest in these two systems.

The techniques for the two-cell systems are much the same. To generate a survival curve for mammary- or thyroid-gland cells in the rat, cells may be irradiated *in vivo* before the gland is removed from donor ani-



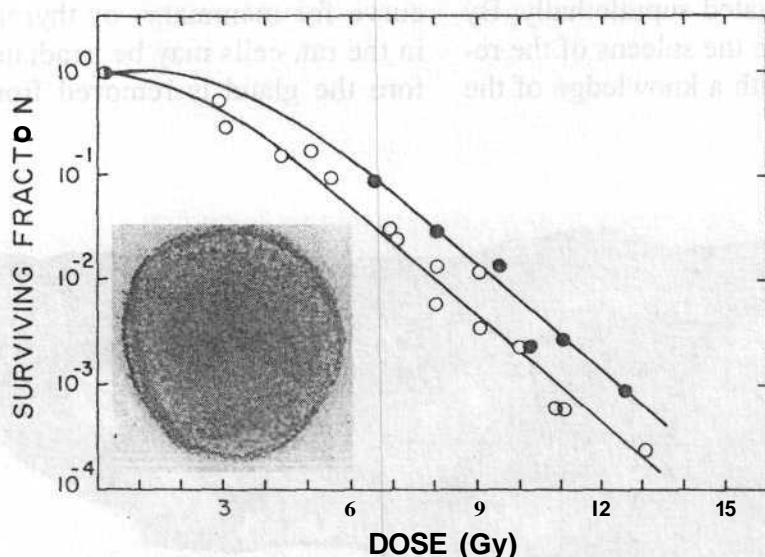
**Figure 18.14.** Photograph of a mouse's spleen. The mouse was irradiated supralethally to sterilize all the cells of the spleen. The nodules of regrowth originate from intravenously injected bone marrow cells from another animal. (Courtesy of Dr. A. Carsten.)



**Figure 18.15.**  $\gamma$ -Ray survival curve for the colony-forming ability of mouse bone marrow cells. The cells are irradiated *in vivo* in the donor animal and grow into colonies in the spleens of supralethally irradiated recipient animals. (Adapted from McCulloch EA, Till JE: Radiat Res 16: 822, 1962, with permission.)

mals and treated with enzymes to obtain a monodispersed cell suspension. Known numbers of cells are injected into the inguinal or interscapular white fat pad of recipient animals.

Under appropriate host conditions and grafted cell numbers, the injection of mammary cells gives rise to mammary structures that are morphologically and functionally normal. One such mammary structure may



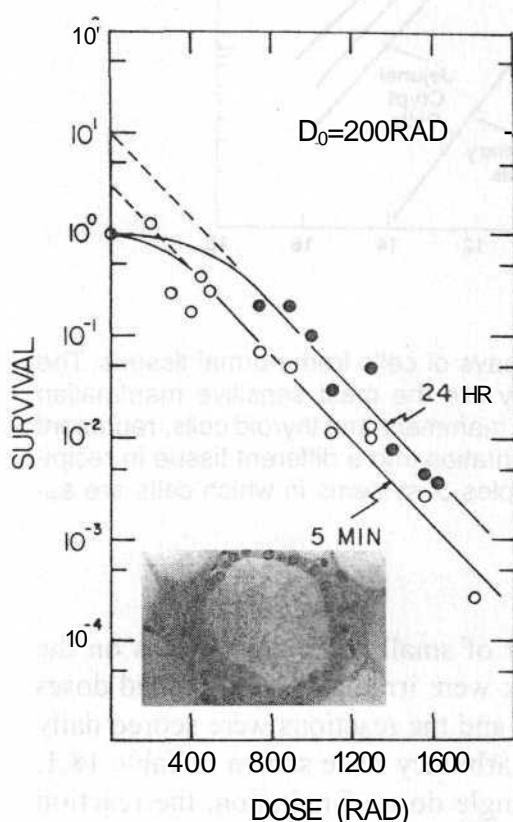
**Figure 18.16.** Dose-response relationship for rat mammary cells assayed by transplantation into the fat pad of recipient animals. (Adapted from Gould MN, Clifton K: Radiat Res 77:149-155, 1979, with permission.) **Inset:** A milk-filled spherical alveolar unit developed from a transplanted cell. (From Gould MN, Biel WF, Clifton KH: Exp Cell Res 107:405-416, 1977, with permission.)

develop from a single cell. By 3.5 weeks after the injection of mammary cells, positive growth is indicated by alveolar units. An example of a milk-filled alveolar unit is shown as an inset in Figure 18.16. If thyroid cells are injected, thyroid follicular units develop (Fig. 18.17).

With either type of cell, a larger number must be injected to produce a growing unit if the cells first are irradiated to a given dose. In practice, some fancy statistics are involved, a discussion of which is beyond the scope of this chapter; in essence, the ratio of the number of irradiated to unirradiated cells required

to produce one growing unit (thyroid follicular unit or alveolar unit) is a measure of the cell-surviving fraction corresponding to the dose. This procedure must be repeated for a range of graded doses to generate a survival curve. The resultant survival curve for mammary cells is shown in Figure 18.16. The characteristics of the curve are unremarkable—the  $D_0$  is about 1.27 Gy (127 rad), and the extrapolation number is about 5, quite typical of rodent cells cultured *in vitro*. The corresponding survival curve for thyroid cells is shown in Figure 18.17. The  $D_0$  is a little larger than for mammary glands assayed in a similar way, implying that the cells are a little more resistant. Figures 18.16 and 18.17 also show data for cells left *in situ* for 24 hours after irradiation before being removed and assayed. If this is done, the shoulder of the survival curve is larger, because of the repair of potentially lethal damage. This is discussed in more detail in Chapter 7.

An interesting use of these clonogen transplant assays is that the physiologic states of either donor or recipient animals can be manipulated hormonally. For the mammary cell assay, cells may be taken from inactive, slowly dividing glands of virgin rats, from rapidly dividing glands of rats in midpregnancy, or from milk-producing glands of lactating rats. For the thyroid cell assay, the physiologic states of both donor and recipient can be manipulated by control of the diet or by partial thyroidectomy.



**Figure 18.17.** Dose-response relationship for rat thyroid cells assayed by transplantation into the fat pad of recipient animals. (From Mulcahy RT, Gould MN, Clifton KH: Radiat Res 84: 523-528, 1980, with permission.) **Inset:** A single thyroid follicle that developed 4 weeks after the inoculation of thyroid cells into the fat pad of recipient animals. (From Clifton KH, Gould MN, Potten CS, Hendry J [eds]: Cell Clones, pp 128-138. New York, Churchill Livingstone, 1985, with permission.)

### SUMMARY OF DOSE-RESPONSE CURVES FOR CLONOGENIC ASSAYS IN NORMAL TISSUES

The survival curves for all of the clonogenic assays in normal tissues are plotted together in Figure 18.18. There is a substantial range of radiosensitivities, with shoulder width being the principal variable. *In vitro* curves for cells from patients with ataxia telangiectasia also are shown, because these are probably the most radiosensitive mammalian cells.

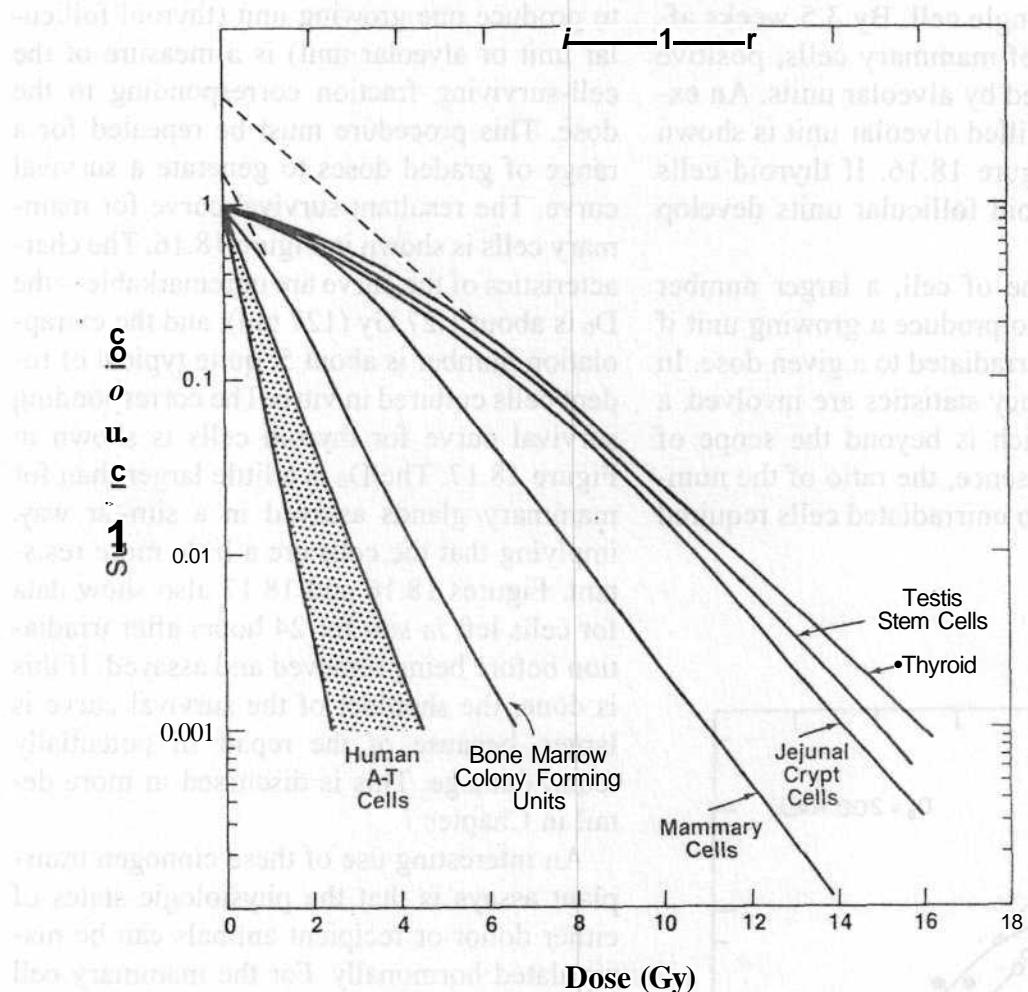


Figure 18.18. Summary of survival curves for clonogenic assays of cells from normal tissues. The human ataxia telangiectasia cells are included because they are the most sensitive mammalian cells. The bone-marrow colony-forming units, together with the mammary and thyroid cells, represent systems in which cells are irradiated and assayed by transplantation into a different tissue in recipient animals. The jejunal crypt and testis stem cells are examples of systems in which cells are assayed for regrowth *in situ* after irradiation.

### DOSE-RESPONSE RELATIONSHIPS FOR FUNCTIONAL ENDPOINTS

#### Pig Skin

Pig skin has been used widely in radiobiologic studies because it has many features in common with human skin, such as color, hair follicles, sweat glands, and a layer of subcutaneous fat. In view of these structural similarities, it is not surprising that the response of pig skin to radiation closely resembles that of human skin, both qualitatively and quantitatively.

Fowler and his colleagues pioneered the use of pig skin as a radiobiologic test system.

A number of small rectangular fields on the pig's flank were irradiated with graded doses of x-rays, and the reactions were scored daily using the arbitrary scale shown in Table 18.1. After a single dose of radiation, the reaction becomes apparent after about 15 days and develops as shown in Figure 18.19.

Two phases of the reaction can be distinguished. First, an early wave of erythema occurred (at 10-40 days), which was variable from one animal to another. This represents the uncomfortable "acute" reaction sometimes seen in patients on radiotherapy at about the end of a course of treatment. Second, a more gradual increase to a second broad wave

**TABLE 18.1.** Radiation Reactions in Pig Skin

Arbitrary Score	Reaction
0	No visible reaction
1	Faint erythema
2	Erythema
3	Marked erythema
4	Moist desquamation of less than half the irradiated area
5	Moist desquamation of more than half the irradiated area

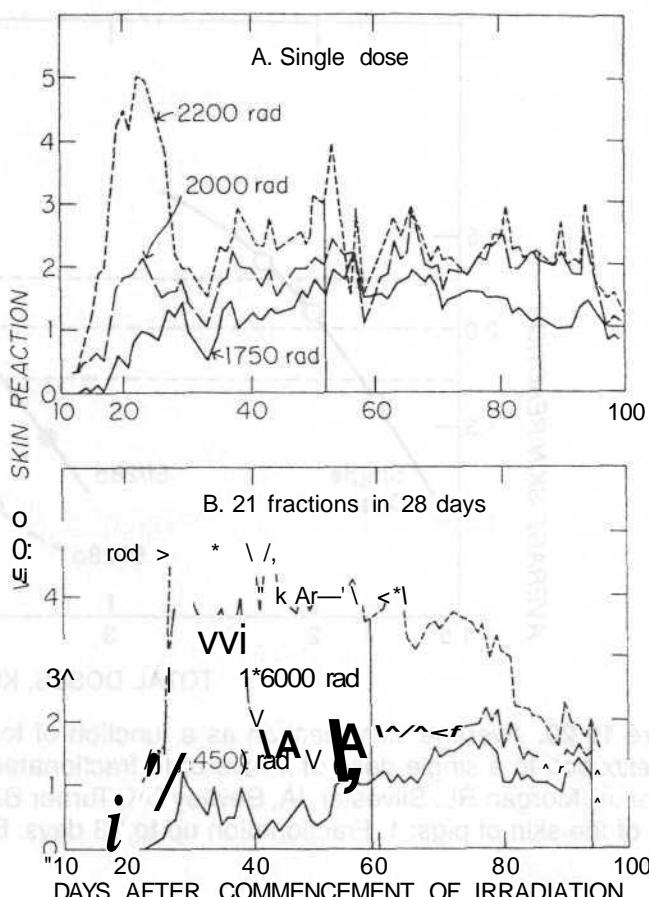
From Fowler JR, Morgan RL, Silvester JA, Bewley DK, Turner BA: Experiments with fractionated x-ray treatment of the skin of pigs: 1. Fractionation up to 28 days. Br J Radiol 36:188-196, 1963, with permission.

of moderately severe reactions took place (between 50 and 100 days), representing a more permanent kind of damage. This second wave shows the tolerance of skin to a more serious type of long-term damage and is also a more repeatable and consistent index of radiation damage. It was subsequently found to correlate well with longer-term damage (up to 2 years) and with subcutaneous damage.

The "score" of radiation damage is taken to be the average skin reaction occurring between certain time limits that encompass the medium-term reactions. After a single dose, this might be a 35-day period between 50 and 85 days after irradiation. For a protracted fractionated regimen, this period of reaction may come later, between days 65 and 100. The average skin reaction in the chosen time period then is plotted as a function of dose; examples of dose-response curves obtained this way are shown in Figure 18.20 for single and fractionated dose schedules.

Late effects also have been studied in pig skin by measuring the contraction that results from fibrosis a year or more after irradiation. A square is tattooed on the skin of the animal in the irradiated field, and the dimensions of this square are recorded as a function of dose as the contraction occurs. This is a primitive but effective measure of late effects.

Many of the important early studies on the fractionation effects of x-rays and the comparison of x-rays with fast neutrons were performed with this biologic system. One over-

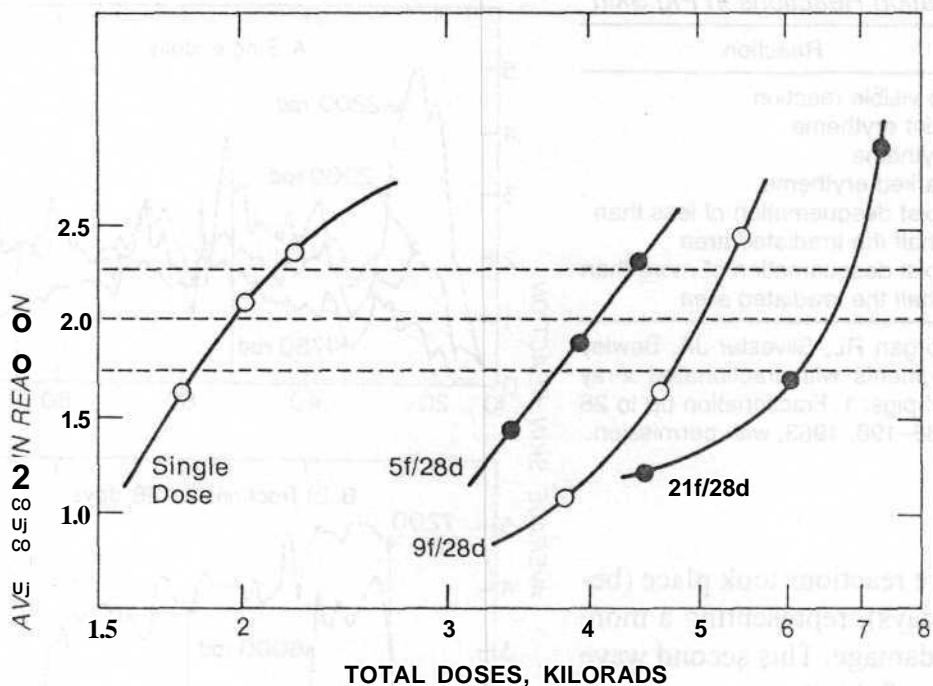


**Figure 18.19.** Development of skin reactions in the pig after graded doses of x-rays, delivered as a single exposure (A) or as multiple fractions spaced over time (B). (Adapted from Fowler JF, Morgan RL, Silvester JA, Bewley DK, Turner BA: Experiments with fractionated x-ray treatment of the skin of pigs: 1. Fractionation up to 28 days. Br J Radiol 36:188-196, 1963, with permission.)

whelming advantage is that data obtained this way can be extrapolated to the human with a high degree of confidence. The balancing disadvantage is that the animals are large and awkward to work with, and their maintenance involves a considerable expense.

### Rodent Skin

Because of the inconvenience and expense of using pigs, the skin of the mouse leg and foot is commonly used instead. One hind leg of each animal is irradiated; the other serves as a control. The skin response is observed each day for about 30 days after irradiation



**Figure 18.20.** Average skin reaction as a function of total dose for medium-term skin reactions in pigs exposed to a single dose of x-rays or to fractionated doses given over 28 days. (Adapted from Fowler JF, Morgan RL, Silvester JA, Bewley DK, Turner BA: Experiments with fractionated x-ray treatment of the skin of pigs: 1. Fractionation up to 28 days. Br J Radiol 36:188-196, 1963, with permission.)

and is scored according to the arbitrary scale shown in Table 18.2. Various doses are used. The progressive development of the reaction after 10 doses of 6 Gy (600 rad) each is illustrated in Figure 18.21; each point represents

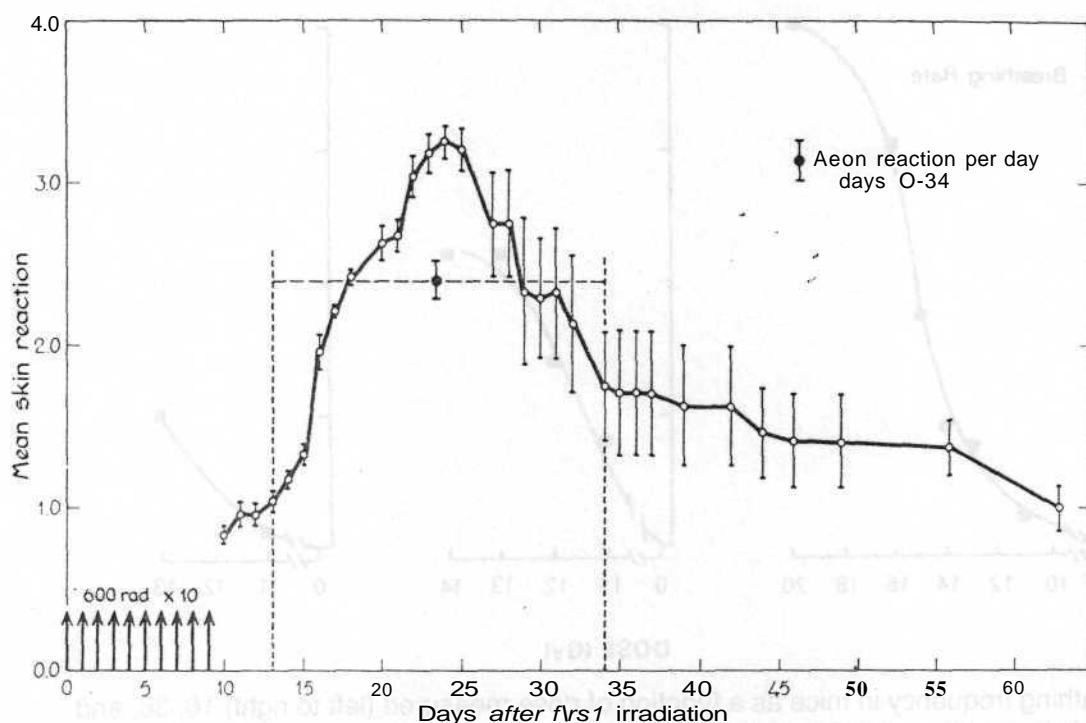
the mean of several animals. Reactions appear by about the 10th day, peak by 15 to 20 days, and then subside. The second wave of the reaction, noted for pig skin, is not seen in mice but is observed in rats.

**TABLE 18.2. Radiation Reactions in Mouse Leg Skin**

Arbitrary Score <sup>a</sup>	Observations
0.5	50/50; doubtful if any difference from normal or not
1-	Because 1 covers a wide range of reddening, even before reaching the severity or additional factors requiring 1+, it is necessary to have 1- for "definite reddening" (i.e., definitely not normal), but only a very slight degree."
1	Definite abnormality; definite reddening, top or bottom of leg; "clean" appearance means not greater than 1
1+	Severe reddening or reddening with definite white marks in creases under foot; query breakdown; query puffiness
1.5	Some breakdown of skin (usually seen on bottom of foot first); scaly or crusty appearance; definite puffiness, plus (query) breakdown; very marked white marks in creases plus puffiness or severe redness
1.5+	Query possibly moist desquamation in small areas
2	Breakdown of large areas of skin or toes stuck together; possibly moist in places but not all moist
2.5	Breakdown of large areas of skin with definite moist exudate
3	Breakdown of most of the skin with moist exudate
3.5	Complete necrosis of limb (rarely seen so far)

<sup>a</sup>+ and - are equivalent to 0.25

From Fowler JF, Kragt K, Ellis RE, Lindop PJ, Berry RJ: Int J Radiat Biol 9:241-252, 1965, with permission.



**Figure 18.21.** Daily skin reaction scores for mice receiving 60 Gy (6,000 rad) in 10 equal fractions to the right hind leg. Each point represents the mean score of six animals; the vertical lines represent the standard errors of the mean. (From Brown JM, Goffinet DR, Cleaver JE, Kallman RF: JNCI 47:75-89, 1971, with permission.)

A dose-response curve is obtained by averaging the skin reaction in the first 30 days and plotting this average as a function of dose.

#### Early and Late Response of the Lung Based on Breathing Rate

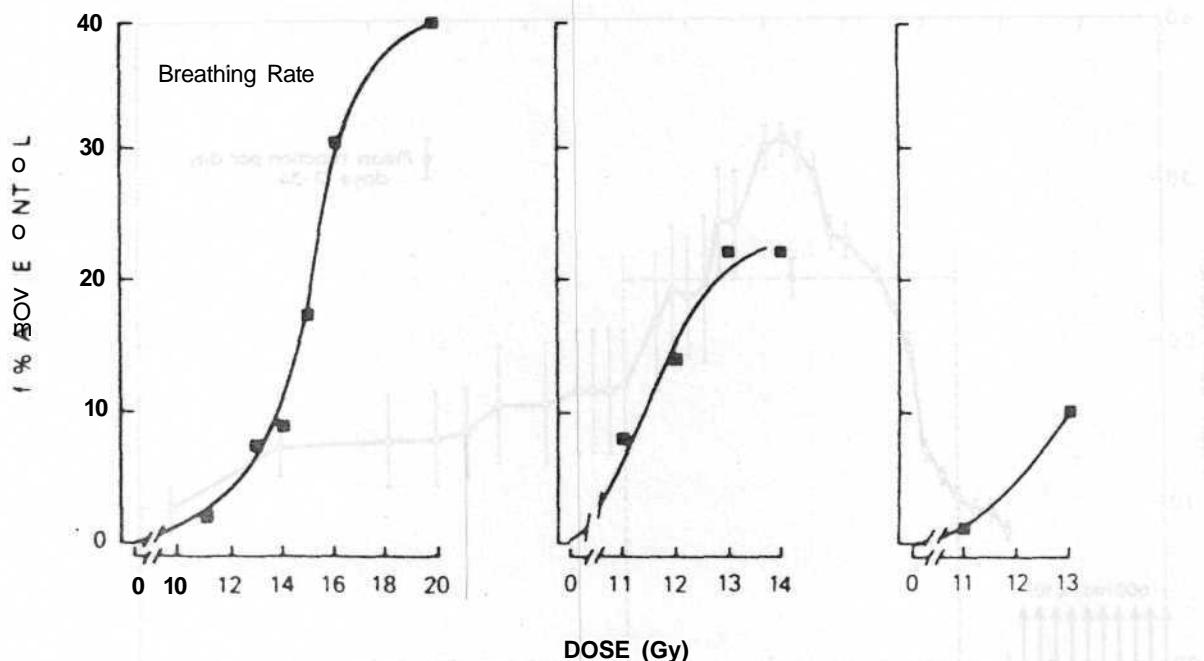
Travis and her colleagues developed a non-invasive assay of breathing frequency to assess both early and late damage in mouse lungs. Breathing frequency increases progressively with dose after a threshold of about 11 Gy (1,100 rad) (Fig. 18.22). The increased breathing frequency in rodent lungs between 14 and 24 weeks is associated with the early response (*i.e.*, pneumonitis); by 52 weeks, the elevated breathing frequency is associated with the late response (*i.e.*, fibrosis). This is a simple but highly quantitative and reproducible system.

#### Spinal-cord Myelopathy

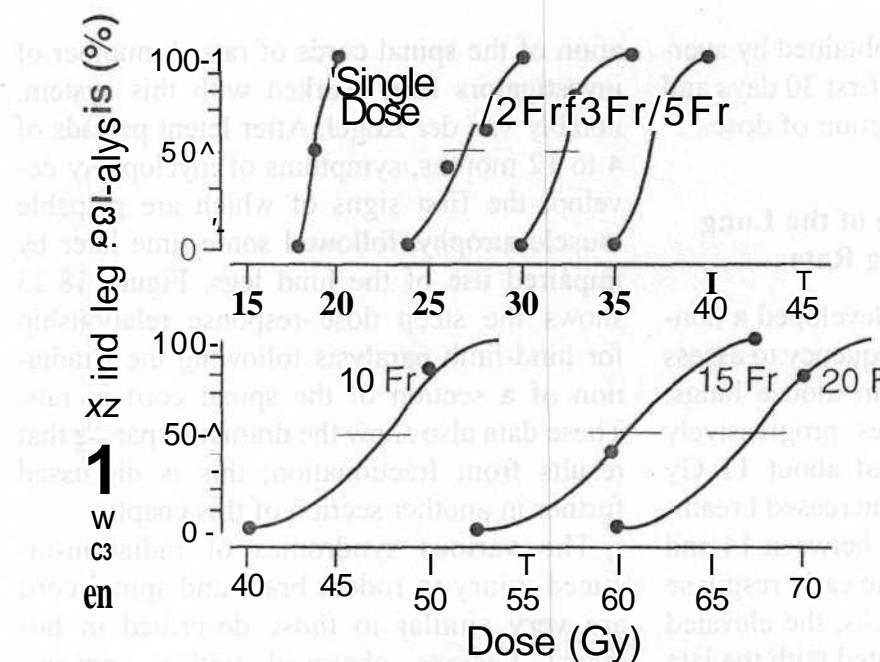
A dose-response relationship can be determined for late damage caused by local irradia-

tion of the spinal cords of rats. A number of investigators have worked with this system, notably van der Kogel. After latent periods of 4 to 12 months, symptoms of myelopathy develop, the first signs of which are palpable muscle atrophy, followed some time later by impaired use of the hind legs. Figure 18.23 shows the steep dose-response relationship for hind-limb paralysis following the irradiation of a section of the spinal cord in rats. These data also show the dramatic sparing that results from fractionation; this is discussed further in another section of this chapter.

The various syndromes of radiation-induced injury in rodent brain and spinal cord are very similar to those described in humans. Lesions observed within approximately the first 6 months after irradiation are limited primarily to the white matter and range between early diffuse or focal demyelination and extensive necrosis. Different pathogenic pathways toward the development of white-matter necrosis have been proposed, with the glial and vascular tissue components



**Figure 18.22.** Breathing frequency in mice as a function of dose measured (left to right) 16, 36, and 52 weeks after irradiation with x-rays. Breathing frequency is expressed as a percentage increase above the age-related control value. (From Travis EL, Down JD, Holmes SJ, Hobson B: Radiation pneumonitis and fibrosis in mouse lung assayed by respiratory frequency and histology. Radiat Res 84:133-142, 1980, with permission.)



**Figure 18.23.** Dose-response curves for the induction of hind-leg paralysis in rats following irradiation of a section of the spinal cord (L2-L5). Note how the dose necessary to produce paralysis increases rapidly with increasing number of fractions. (Redrawn from van der Kogel AJ: Late Effects of Radiation on the Spinal Cord, pp 1-160. Rijswik, The Netherlands, The Radiobiological Institute of The Organization for Health Research TNO, 1979, with permission.)

the major targets. The most common type of late delayed injury peaks at 1 to 2 years postirradiation and almost certainly has a vascular basis. Another type of late injury that has been described more recently in various species, including humans, is slowly progressive glial atrophy. This lesion is not associated with necrosis but occurs diffusely and at lower doses. With improvements in diagnostic procedures such as magnetic resonance imaging, glial atrophy may become a more frequently recognized adverse effect of brain tumor therapy.

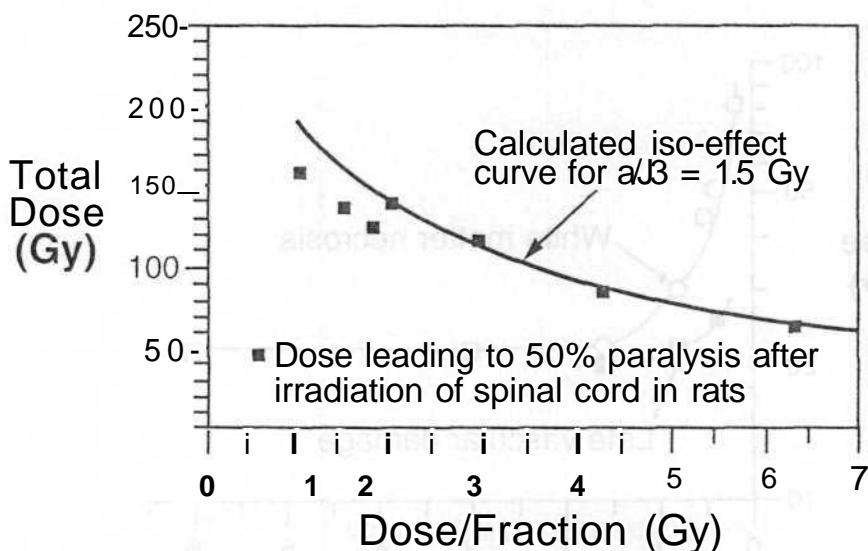
#### *Latency*

Over a dose range of about 25 to 60 Gy (2,500-6,000 rad), delivered in single doses, the general tendency is a decreasing latency with increases in dose of approximately 2 days/Gy (2 days/100 rad). There is a considerable variation with animal strain, as well as with the region of the cord irradiated. In terms of mechanisms, demyelination or slowly progressive atrophy is probably a consequence of

interference with the slow continuous turnover of oligodendrocytes by killing of glial progenitor cells. Vascular injury may accelerate, precipitate, or even initiate the white-matter changes leading to necrosis. This is an area of some controversy.

#### *Fractionation and Protraction*

The effect of dose fractionation and protraction on tolerance to radiation has been investigated extensively in the rat spinal cord, and to a lesser extent in the mouse, monkey, and guinea pig. Because these systems turn over slowly, there is little influence of overall treatment time up to any conventional clinical regimen of 6 to 8 weeks. On the other hand, dose per fraction is very important (Fig. 18.23), with the dose to produce paralysis increasing dramatically with number of fractions. The effect of a large number of very small fractions also has been investigated. Figure 18.24 shows the relation between total dose and dose per fraction to produce paralysis in 50% of rats from irradiation of a short



**Figure 18.24.** The data points show total dose, as a function of dose per fraction, to produce paralysis in 50% of rats after irradiation of the spinal cord. The curve is an iso-effect relationship based on the linear-quadratic equation with an  $a/p$  of 1.5 Gy. The experimental data suggest that the linear-quadratic model overestimates tolerance for dose per fraction values less than 2 Gy. This may be a result of incomplete repair, because the interfraction interval was only 4 hours. (Adapted from van der Kogel AJ: Central nervous system radiation injury in small animal models. In Gutin PH, Leibel SA, Sheline GE [eds]: Radiation injury to the Nervous System, pp 91-112. New York, Raven Press, 1991, with permission.)

length of cervical spine. The smooth curve is an isoeffect curve calculated for the very low  $a/[3]$  value of 1.5 Gy (150 rad). The experimental data suggest that the linear-quadratic (LQ) model overestimates the tolerance for small doses per fraction of less than 2 Gy (200 rad). However, this may be a result of incomplete repair, because in these experiments the interfraction interval was only 4 hours. There is good reason to believe that repair of sublethal damage takes place slowly in this normal tissue, and indeed repair may be biphasic, with "fast" and "slow" components. For this reason, if multiple doses per day are used to the spinal cord, the interfraction interval should be at least 6 to 8 hours.

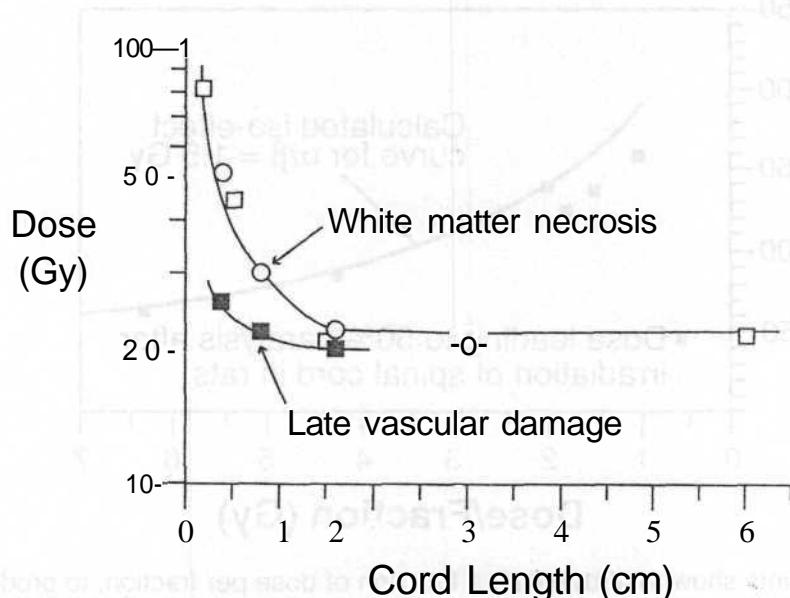
#### Volume Effects

The total volume of irradiated tissue usually is assumed to have an influence on the development of tissue injury. The spinal cord is, perhaps, the clearest case in which the functional subunits (FSUs) are arranged in linear fashion like links in a chain. Figure 18.25 shows the relation between tolerance dose and

the length of cord irradiated in the rat. For short lengths of cord, below 1 cm, tolerance in terms of white-matter necrosis shows a marked dependence on the length of cord irradiated. Late vascular injury shows less dependence on cord length. Beyond a few centimeters, the tolerance is virtually independent of the length of cord irradiated. This would be predicted from the linear arrangement of the functional subunits. A chain is broken whether one, two, three, or more links are removed.

#### *Re-treatment after Long Time Intervals*

The spinal cord does recover to some extent after long time periods following irradiation. The extent of the recovery depends, of course, on the first treatment, that is, what fraction of tolerance was involved. Experiments with rats indicate that, after an initial treatment to 50% tolerance, the re-treatment tolerance approaches 90% of the tolerance of the untreated control group by about a year after the initial irradiation. If the initial treatment represented a larger fraction of tolerance, the re-treatment that can be tolerated is reduced.



**Figure 18.25.** The dependence of spinal-cord tolerance on the length of cord irradiated in the rat. For short lengths of cord, shorter than about 1 cm, tolerance for white-matter necrosis shows a marked dependence on the length of cord irradiated. Beyond a few centimeters, the tolerance dose is virtually independent of the length of cord irradiated. (Adapted from van der Kogel AJ: Central nervous system radiation injury in small animal models. In Gutin PH, Leibel SA, Sheline GE (eds): Radiation Injury to the Nervous System, pp 91-112. New York, Raven Press, 1991, with permission.)

### INFERRING THE RATIO $\alpha/\rho$ FROM MULTIFRACTION EXPERIMENTS IN NONCLONOGENIC SYSTEMS

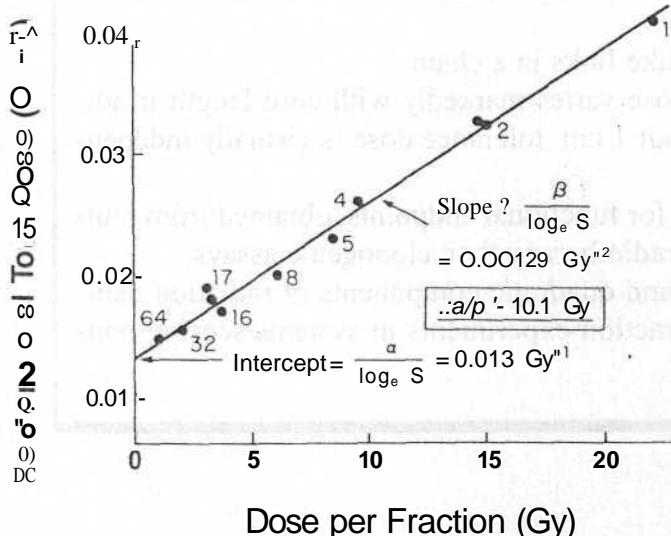
The parameters of the dose-response curve for any normal tissue system for which a functional endpoint can be observed may be inferred by performing a multifraction experiment. Take, for example, an experiment in which mouse foot skin reaction is scored. Doses that result in the same skin reaction (*e.g.*, moist desquamation over 50% of the area irradiated) if delivered as a single exposure in a multifraction regimen (*e.g.*, 5, 10, or 20 fractions) must be determined experimentally. A number of assumptions must be made:

1. The dose-response relationship is represented adequately by the linear-quadratic formulation:

$$S = e^{-\alpha D - \beta D^2}$$

in which  $S$  is the fraction of cells surviving in a dose,  $D$ , and  $\alpha$  and  $\beta$  are constants.

2. Each dose in a fractionated regimen produces the same biological effect.
3. Full repair of sublethal damage takes place between dose fractions; but no cell proliferation occurs. Suppose the total dose,  $D$ , is divided into  $n$  equal fractions of dose  $d$ . The previous equation then can be rewritten:



$$S \equiv (e^{-ad} - \beta d^2)^n$$

or

$$-\log_e S / nd = a + (\beta d)$$

If the reciprocal of the total dose ( $1/nd$ ) is plotted against the dose per fraction ( $d$ ), a straight line results, as shown in Figure 18.26. The intercept on the ordinate gives  $a/\log_e S$ ; the slope gives  $\beta/\log_e S$ . In general, the value of  $\log_e S$  is not known unless other cell-survival studies are available, but the ratio of the intercept to the slope provides an estimate of  $a/p$ .

Multifraction experiments have been performed and estimates of  $a/p$  made for essentially all of the normal-tissue endpoints described in this chapter. One of the important conclusions arrived at is that the value of  $a/p$  tends to be larger for early-responding tissues, about 10 Gy (1,000 rad), than for late-responding tissues, about 2 Gy (200 rad).

Because  $a/p$  is the dose at which cell killing by linear and by quadratic components are equal (Chapter 3), the implication is that dose-response relationships for late-responding tissues are "curvier" than for early-responding tissues. The importance of this conclusion becomes evident in the discussion of fractionation in radiotherapy in Chapter 22.

**Figure 18.26.** Reciprocal of the total dose required to produce a given level of injury (acute skin reaction in mice) as a function of dose per fraction in multiple equal doses. The overall time of these experiments was sufficiently short so that proliferation could be neglected; numbers of fractions are shown by each point. From the values of the "intercept" and "slope" of the best-fit line, the values of  $a$  and  $\beta$  and the ratio  $a/p$  for the dose-response curve for organ function can be determined. (Adapted from Douglas BG, Fowler JR: Radiat Res 66:401, 1976, with permission.)

### SUMMARY OF PERTINENT CONCLUSIONS

- After irradiation, most cells die a **mitotic death**; that is, they die in attempting the next or a later mitosis. In some tissues cells die by apoptosis, which is a programmed cell death.
- Systems involving clonogenic endpoints (*i.e.*, cell survival) for cells of normal tissues include some in which cells regrow *in situ* and some in which cells are transplanted to another site.
- *In situ* regrowth techniques include the skin, crypt cells in the jejunum or colon, testes stem cells, and kidney tubules. Single-dose experiments can yield the slope (Do) of the dose-response curve over a range of high doses. Multifraction experiments allow the whole dose-response curve to be reconstructed.
- Systems in which cell survival is assessed by transplantation into another site include bone-marrow stem cells, thyroid cells, and mammary cells.
- A dose-response curve for bone-marrow stem cells can be obtained by allowing cells from the donor animal to lodge and grow in the spleen of recipient animals. These are the most sensitive normal mammalian cells that die a mitotic death; Do is close to 1 Gy (100 rad), with little or no shoulder.
- Dose-response curves for mammary and thyroid cells can be obtained by transplanting them into a fat pad of recipient animals.
- The radiosensitivity of cells from normal tissues varies widely. The width of the shoulder of the curve is the principal variable. Jejunal crypt cells have a very large shoulder; bone-marrow stem cells have little, if any, shoulder. Most other cell types studied in clonogenic assays fall in between.
- Dose-response curves for functional endpoints, distinct from cell survival, can be obtained for:
  1. Pig skin and rodent skin by measuring skin reactions
  2. Early and late response of the lung by measuring breathing rate
  3. Spinal cord by observing myelopathy
    - a. Paralysis develops after a latency of months to years
    - b. Early lesions limited to white matter; late delayed injury probably has a vascular basis
    - c. Spinal cord damage very sensitive to fractionation; o/c/p of about 1.5 Gy (**150** rad)
    - d. Sublethal damage repair probably has "fast" and "slow" components
    - e. If multiple fractions per day are used, interfraction interval should be at least 6 to 8 hours
    - f. Functional subunits arranged serially like links in a chain
    - g. For short lengths of cord, tolerance dose varies markedly with cord length irradiated; for cord lengths greater than about 1 cm, tolerance dose is virtually independent of cord length
- The shape of the dose-response relationship for functional endpoints, obtained from multifraction experiments, is more pertinent to radiotherapy than clonogenic assays.
- The ratio  $\alpha/3$  (the dose at which the linear and quadratic components of radiation damage are equal) may be inferred from multifraction-experiments in systems scoring non-clonogenic endpoints.

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# 19

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## Clinical Response of Normal Tissues

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### CELLS AND TISSUES

#### EARLY (OR ACUTE) AND LATE EFFECTS

#### FUNCTIONAL SUBUNITS IN NORMAL TISSUES

#### THE VOLUME EFFECT IN RADIOTHERAPY:

##### TISSUE ARCHITECTURE

#### RADIATION PATHOLOGY OF TISSUES

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### CASARETT'S CLASSIFICATION OF TISSUE

#### RADIOSENSITIVITY

#### MICHALOWSKI'S H AND F-TYPE POPULATIONS

#### GROWTH FACTORS

#### SPECIFIC TISSUES AND ORGANS

#### SUMMARY OF PERTINENT CONCLUSIONS

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### CELLS AND TISSUES

The cells of normal tissues are not independent but form a complete integrated structure. There is a delicate balance between cell birth and cell death to maintain tissue organization and the number of cells in it. The response to damage is governed by the inherent cellular radiosensitivity, the kinetics of the tissue, and the way in which cells are organized in that tissue.

If the fate of individual cells is studied, as described in Chapter 3, there is a continuous monotonic relationship between the magnitude of the dose and the fraction of cells that are "killed," in the sense that they lose their reproductive integrity—the ability to divide indefinitely. By contrast, no effects are seen in tissues after small doses, though effects of increasing severity become apparent if the dose rises above a threshold level. The reason is, of course, that killing a small number of cells in a tissue matters very little; visible damage is evident only if a large enough proportion of the cells are killed and removed from the tissue. The threshold dose below which no effect is seen and the delay between irradiation and the time at which the **damage**

becomes observable vary greatly among different tissues.

Cell death after irradiation occurs mostly as cells attempt to divide. In tissues with a rapid turnover rate, damage becomes evident quickly; in a matter of hours in the intestinal epithelium and bone marrow, in a matter of days in the skin and mucosa. In tissues in which cells divide rarely, radiation damage to cells may remain latent for a long period of time and be expressed very slowly. Radiation damage to cells that are already on the path to differentiation, and were not planning to divide many times anyway, is of little consequence. By contrast, radiation damage to stem cells has serious repercussions because they were programmed to divide many times to maintain a large population, and if they lose their reproductive integrity both they and their potential descendants are lost from the population. Thus cells on the road to differentiation appear to be more radioresistant than stem cells. In fact, the fraction of cells surviving a given dose may be identical at the single cell level, so strictly speaking it is their **radioresponse** that is different, not their **radiosensitivity**. This explains the so-called law of Bergonie and Tribondeau (1906), who noted

that tissues appear to be more "radiosensitive" if their cells are less-well differentiated, have a greater proliferative capacity, and divide more rapidly.

### EARLY (OR ACUTE) AND LATE EFFECTS

Radiation effects are commonly divided into two categories, **early** and **late**, which show quite different patterns of response to fractionation; their dose-response relations are characterized by different  $\alpha/\beta$  ratios, as described in more detail in Chapter 22. **Late effects** are much more sensitive to changes in fractionation than early effects. **Early, or acute, effects** result from the death of a large number of cells and occur within a few days or weeks of irradiation in tissues with a rapid tumor rate. Examples include effects in the epidermal layer of the skin, gastrointestinal epithelium, and hematopoietic system, in which the response is determined by a hierarchical cell lineage, composed of stem cells and their differentiating offspring. The time of onset of early reactions correlates with the relatively short lifespan of the mature functional cells; the identity of the target cells is usually obvious.

Late effects appear after a delay of months or years and occur predominantly in slowly proliferating tissues such as lung, kidney, heart, liver, and central nervous system. The difference between the two types of lesions lies in their progression: Acute damage is repaired rapidly, because of the rapid proliferation of stem cells, and may be completely reversible. By contrast, late damage may improve but is never completely repaired. A late effect may result from a combination of vascular damage and loss of parenchymal cells. Clearly, vascular damage is not the dominant factor in **every** case, because if it were, the dose-effect relationship would be the same for all tissues, and that is not the case. This may be true for some tissues, however, including the spinal cord. If intensive fractionation protocols deplete the stem cell population below levels needed for tissue

restoration, an early reaction in a rapidly proliferating tissue may persist as a chronic injury. This has been termed a *consequential late effect*, that is, a late effect consequent to, or evolving out of, a persistent severe early effect.

### FUNCTIONAL SUBUNITS IN NORMAL TISSUES

The fraction of cells surviving determines the success or failure of a treatment regimen as far as the tumor is concerned, because a single surviving cell may be the focus for the regrowth of the tumor. For normal tissues, however, it is not the whole story. The tolerance of normal tissues for radiation depends on the ability of the clonogenic cells to maintain a sufficient number of mature cells suitably structured to maintain organ function. The relationship between the survival of clonogenic cells and organ function, or failure, depends on the structural organization of the tissue. Many tissues may be thought of as consisting of **functional subunits** (FSUs).

In some tissues, the FSUs are discrete, anatomically delineated structures whose relationship to tissue function is clear. Obvious examples are the nephron in the kidney, the lobule in the liver, and perhaps the acinus in the lung. In other tissues, the FSUs have no clear anatomic demarcation. Examples include the skin, the mucosa, and the spinal cord. The response to radiation of these two types of tissue—with structurally defined or structurally undefined FSUs—is quite different.

The survival of **structurally defined** FSUs depends on the survival of one or more clonogenic cells within them, and tissue survival in turn depends on the number and radiosensitivity of these clonogens. Although such tissues are composed of a large number of FSUs, each is a small self-contained entity independent of its neighbors. Surviving clonogens cannot migrate from one to the other. Because each FSU is both small and autonomous, low doses deplete the clonogens in

it. Each kidney, for example, is composed of a large number of relatively small FSUs, each of which is a self-contained structural entity independent of its neighbors. Consequently, survival of a nephron after irradiation depends on the survival of at least one clonogen within it, and therefore on the initial number of renal tubule cells per nephron and their radiosensitivity. Because this FSU is relatively small, it is completely depleted of clonogens by low doses, which accounts for the low tolerance to radiation of the kidney. Other organs that resemble the kidney in having structurally defined FSUs not repopulated from adjacent FSUs may be those with a branching tree-like system of ducts and vasculature that ultimately terminate in "end structures" or lobules of parenchymal cells. These can be visualized as independent structurally defined FSUs. Examples of organs with this tissue architecture include the lung, liver, and exocrine organs. At least some of these also have low tolerance to radiation.

By contrast, the clonogenic cells that can repopulate the **structurally undefined FSUs** after depletion by radiation are not confined to one particular FSU itself. Rather, clonogenic cells can migrate from one FSU to another and allow repopulation of a depleted FSU. For example, reepithelialization of a denuded area of skin can occur either from surviving clonogens within the denuded area or by migration from adjacent areas. A concept proposed to link the survival of clonogenic cells and functional survival is the tissue-rescue unit, defined to be the minimum number of FSUs required to maintain tissue function. This model assumes that the number of tissue rescue units in a tissue is proportional to the number of clonogenic cells, that FSUs contain a constant number of clonogens, and that FSUs can be repopulated from a single surviving clonogen.

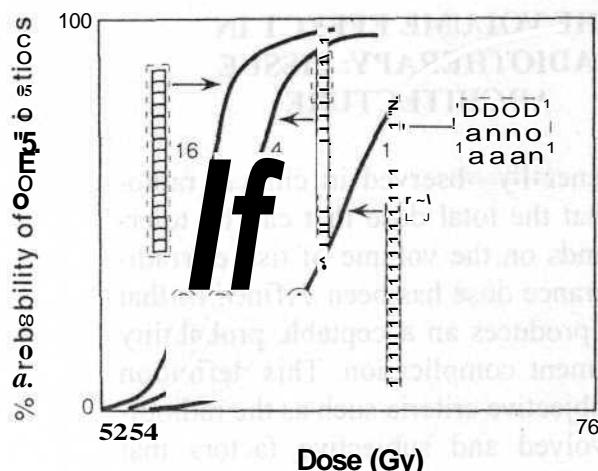
Some tissues defy classification by this system. The crypts of the jejunum, for example, are structurally well-defined subunits, but surviving crypt cells can and do migrate from one crypt to another to repopulate depleted neighbors.

## THE VOLUME EFFECT IN RADIOTHERAPY: TISSUE ARCHITECTURE

It is generally observed in clinical radiotherapy that the total dose that can be tolerated depends on the volume of tissue irradiated. Tolerance dose has been defined as that dose that produces an acceptable probability of a treatment complication. This definition includes objective criteria such as the radiobiology involved and subjective factors that may be socioeconomic, medicolegal, or psychological.

The spatial arrangement of the FSUs in the tissue is critical. In the case of tissues in which the FSUs are arranged in series, like the links of a chain, the integrity of each is critical to organ function, and elimination of any one results in a measurable probability of a complication. The spinal cord is the clearest example in which specific functions are controlled by specific segments arranged linearly. Because impulses must pass along the cord, death of critical cells in any one segment results in complete failure of the organ. The effect on the probability of development of a threshold-binary or quantum response in a serial FSU tissue is illustrated in Figure 19.1. As the field size increases to include a greater number of FSUs, 1, 4, or 16 in this example, the curve relating probability of a complication to dose rises much more steeply with dose and moves to lower doses. This explains the important volume effect found **in**, for example, the spinal cord, **in** which FSUs are arranged in series and loss of any one may result in myelopathy.

By contrast, tissues in which the FSUs are not arranged serially show a graded dose response and do not show a volume effect at lower levels of injury at which healing can occur from surviving clonogens scattered throughout the treatment volume. This would be true for the skin or mucosa, **in** which a volume effect would not be expected on radiobiologic grounds. This is never quite true in practice, because if a larger area of skin or mucosa is ulcerated, the prolonged healing



**Figure 19.1.** Relationship between dose and probability of complications for different types of normal tissues. Curve A relates to a normal tissue in which the functional subunits are not arranged serially regardless of whether one or all subunits are exposed (*i.e.*, regardless of field size). It also applies to a normal tissue in which functional subunits are arranged serially if only one subunit is exposed (*i.e.*, if the field is small). Note that the curve is relatively shallow (*i.e.*, the probability of a complication rises relatively slowly with dose). Curves B and C refer to a tissue with serially arranged functional subunits; the complication curve gets steeper and moves to lower doses as the treatment field size increases. For example, curves B and C relate to 4 or 16 functional subunits exposed. (Note that the position of the curves in relation to the abscissae is arbitrary, resulting from two assumptions: an effective  $D_0$  of 4 Gy for a survival curve for cells exposed to multiple doses of 2 Gy, and that 58 Gy in 2-Gy fractions sterilizes 10% of the functional subunits.) (Adapted from Withers HR, Taylor JMG, Maciejewski B: Treatment volume and tissue tolerance. *Int J Radiat Oncol Biol Phys* 14:751-759, 1988, with permission.)

time plus the increased potential for infection are more debilitating than similarly severe ulceration in a smaller area. In other words, although the severity of a skin reaction is relatively independent of the area irradiated, because healing occurs by regeneration of surviving clonogens scattered throughout the treated area, the tolerability is not. Therefore, there is a volume effect in clinical practice, but it is not based on an increased probability of injury as it is in tissues in which FSUs are arranged serially.

## RADIATION PATHOLOGY OF TISSUES

The response of a tissue or organ to radiation depends primarily on three factors: (1) the inherent sensitivity of the individual cells; (2) the kinetics of the population as a whole of which the cells are a part; and (3) the way in which cells are organized in that tissue. These factors combine to account for the substantial variation in response to radiation characteristic of different tissues.

In the case of tissues composed of highly differentiated cells that are performing specialized functions, cell-survival curves are largely irrelevant, because these cells have no mitotic future. Little information is available at the cellular level concerning the effects of radiation on differentiated cells. All that can be said is that, in general, the amount of radiation needed to destroy the functioning ability of a differentiated cell is far greater than that necessary to stop the mitotic activity of a dividing cell.

A closed static population, composed entirely of mature differentiated cells, therefore, is very resistant to radiation. In the case of self-renewing tissues, the Achilles heel is the dividing cell: Loss of reproductive ability in an appreciable fraction of these cells occurs after a moderate dose of a few grays (a few hundred rads). Whether the tissue or organ as a whole appears to be affected to a small or large extent—and is consequently labeled as sensitive or resistant—depends on the extent to which the tissue involved can continue to function adequately with a reduced number of cells.

Another factor that is evident from even this most elementary consideration of population kinetics is that the time interval between the delivery of the radiation insult and its expression in tissue damage is very variable for different populations. This time interval is determined by the normal lifespan of the mature functional cells and the time it takes for a cell "born" in the stem cell compartment to mature to a functional state. For example, mature erythrocytes in circulating blood have a relatively long lifespan and are separated from the

primitive stem cell compartment by a number of transit compartments, so that time is required for a cell to pass through the various stages of differentiation and maturation. Consequently, a considerable time interval elapses between the depopulation of the stem cell compartment and the final expression of this injury in terms of a reduced peripheral blood cell count. By contrast, in the case of the intestinal epithelium, the mature functional cells on the surface of the villi have a short lifespan, and the time interval between the "birth" of a new cell in the stem compartment of the crypt and its appearance as a mature functional cell is very short, on the order of a few days. As would be expected, therefore, radiation damage is expressed correspondingly quickly in this tissue. Two systems commonly are used to classify tissue radiosensitivity in terms of population kinetics and tissue architecture.

### CASARETT'S CLASSIFICATION OF TISSUE RADIOSENSITIVITY

The limitations of our knowledge of cellular population kinetics is remedied to some extent by a wealth of information on the relative sensitivities of various tissues based on histopathologic observations. It must be emphasized that these data are based on entirely

different endpoints than those with which previous chapters have been concerned. To score a cell as "dead" by observing a fixed and stained section of tissue through a microscope is quite different from the experimental test of cell death in terms of loss of reproductive capacity, which has been used previously. Nevertheless, the study of radiation pathology provides data that are highly relevant to clinical radiotherapy.

Casarett has suggested a classification of mammalian cell radiosensitivity based on histologic observation of early cell death. He divided parenchymal cells into four major categories, numbered I through IV (Table 19.1). The supporting structures, such as the connective tissue and the endothelial cells of small blood vessels, are regarded as intermediate in sensitivity between groups II and III of the parenchymal cells.

One of the most sensitive cells to radiation in fact defies all the "laws" and systems of classification; it is the small lymphocyte. This cell, it is believed, never divides at all, or at least only in exceptional circumstances. Small lymphocytes disappear from circulating blood after very small doses of radiation, and it is believed that they suffer an interphase death (by the process of apoptosis). Most sensitive cells die a mitotic death after irradiation; most cells that never divide require very large

TABLE 19.1. *Categories of Mammalian Cell Sensitivity*

Cell Type	Properties	Examples	Sensitivity <sup>a</sup>
I Vegetative intermitotic cells	Divide regularly; no differentiation	Erythroblasts Intestinal crypt cells Germinal cells of epidermis Myelocytes	High
II Differentiating intermitotic cells	Divide regularly; some differentiation between divisions		
Connective tissue cells <sup>b</sup>			
III Reverting postmitotic cells	Do not divide regularly; variably differentiated	Liver	
IV Fixed postmitotic cells	Do not divide; highly differentiated	Nerve cells Muscle cells	Low

<sup>a</sup>Sensitivity decreases for each successive group.

<sup>b</sup>Intermediate in sensitivity between groups II and III.

Based on Casaret G in Harris RJC (ed): Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiations. New York, Academic Press, 1963; and Rubin R. Casarett GW: Clinical Radiation Pathology, vol 1. Philadelphia, WB Saunders, 1968, with permission.

doses to kill them. The small lymphocyte breaks both of these rules, inasmuch as it does not usually divide, dies of interphase death, and at the same time is one of the most sensitive mammalian cells.

Group I of Casarett's classification, the most sensitive group, includes the stem cells of the classic self-renewing systems, such as the basal layers of the epidermis of the skin and the intestine, the erythroblasts or precursors of the red blood cell, and the primitive cells of the spermatogenic series. The stem cells divide regularly and provide a steady and abundant supply of progeny, some of which differentiate and mature into functioning cells. A reservoir of primitive dividing stem cells is maintained and in some cases can be triggered to divide more rapidly in response to a need. The primitive dividing stem cells are vulnerable to radiation; a moderate dose causes a proportion of them to "die" in attempting the next or a subsequent mitosis. The time of crisis for the organism as a whole occurs if the supply of functioning cells is inadequate: a shortage of circulating red and white blood cells in the case of the blood, and a shortage of mature covering dermal cells in the case of the skin. The time interval between irradiation and the crisis is about equal to the lifespan of the mature functioning cells. As the functioning cells die off at the end of their natural lifespans, there are none to take their place if a dose of radiation previously has depopulated the stem cell compartment. Depending on the size of the dose, the organ or tissue may not survive the critical time at which the number of functioning cells reaches a minimum value.

Group II consists of cells that divide regularly but that also mature and differentiate between divisions. Cells in this category are relatively short-lived as individuals and are produced by division of vegetative intermitotic cells. These cells usually complete a limited number of divisions and differentiate to some extent between successive mitoses. This group includes cells of the hematopoietic series in the intermediate stages of differentiation and likewise the more differentiated spermatogonia and spermatocytes.

Group III, the reverting postmitotic cells, are relatively resistant and as individuals have relatively long lives. Ordinarily they do not undergo mitosis, but they are capable of dividing with the appropriate stimulus, which is usually damage or loss of many of their own kind. The liver cells are a good example of cells of this category. In the adult, there is normally little or no cell division, but if a large part of the liver is removed by surgery, the remaining cells are triggered to divide and make good the loss. Other examples in this category include the cells of the kidney and pancreas and of various glands, such as the adrenal, thyroid, and pituitary.

Group IV consists of the fixed postmitotic cells. These generally are considered to be the most resistant to radiation. They are highly differentiated and appear to have lost the ability to divide. Some have a long lifespan, such as the neurons. Others have a short lifespan, such as the granulocytes, which have to be continually replaced by the division of more primitive cells. The superficial epithelial cells of the gut also fall into this category. In the normal course of events they are sloughed off the tops of the villi and replaced by cells dividing in the crypts.

#### MICHALOWSKI'S H-AND F-TYPE POPULATIONS

Within tissues, three distinct categories of cells can be identified. First, **stem cells**, which are capable of unlimited proliferation and escape senescence because of telomere shortening by activating the enzyme telomerase. An example includes the crypt cells in the intestinal mucosa. The cells produced by stem cell proliferation both maintain the stem cell pool and provide candidates for differentiation. Second, at the other extreme, are **functional cells** that are fully differentiated; they are usually incapable of further division and die after a **finite** lifespan, though the lifespan varies enormously between different cell types. Examples include circulatory granulocytes and the cells that make up the villi of the intestinal mucosa. Between

these two extremes are **maturing** partially differentiated cells; these are descendants of the stem cells, still multiplying as they complete the process of differentiation. In the bone marrow, for example, the erythroblasts and granuloblasts represent intermediate compartments.

Many tissues are examples of this **hierarchical** model (**H-type** populations), including the hematopoietic bone marrow, intestinal epithelium, and epidermis. Other tissues, such as liver, thyroid, and dermis, are composed of cells that rarely divide under normal conditions but can be triggered to divide by damage to the tissue or organ. "**Flexible**" tissues (**F-type** populations) have no compartments and no strict hierarchy. After damage to the tissue, all cells including those that are functional enter the cell cycle. The time interval before damage becomes evident is a function of dose. If the dose is small, the expression of damage is delayed because cells divide infrequently. Consequently, the damage may be hidden for a long time.

Many tissues are hybrids of these two extreme models, with most cells able to make a few divisions and a minority of the population behaving as stem cells.

## GROWTH FACTORS

Radiation induces interleukins 1 as well as 6. Interleukin 1 acts as a radioprotectant of hematopoietic cells by increasing both the shoulder and the Do of the survival curve. **Basic fibroblast growth factor** induces endothelial cell growth, inhibits radiation-induced apoptosis, and therefore protects against microvascular damage. This growth factor is produced in response to stress (heat, hypoxia, chemicals, radiation) and tends to reduce late effects. Microvascular protection is more effective in branching midsize capillaries (in which higher concentrations of basic fibroblast growth factor are seen) than in nonbranching capillaries. To the extent that radiation-induced late effects are mediated by damage to blood vessels, radiation tolerance is high in organs with large blood vessels

(corresponding to high levels of growth factors) and lower near nonbranching capillaries.

**Platelet derived growth factor**  $\beta$  increases damage to vascular tissue. **Transforming growth factor beta** induces a strong inflammatory response, for example in pneumonitis. It stimulates the growth of connective tissue and tends to inhibit epithelial cell growth. Consequently, fibrosis and vascular changes associated with late radiation effects are associated with this factor. Transforming growth factor beta may down-regulate interleukin 1 and tumor necrosis factor and increase damage to hematopoietic tissue.

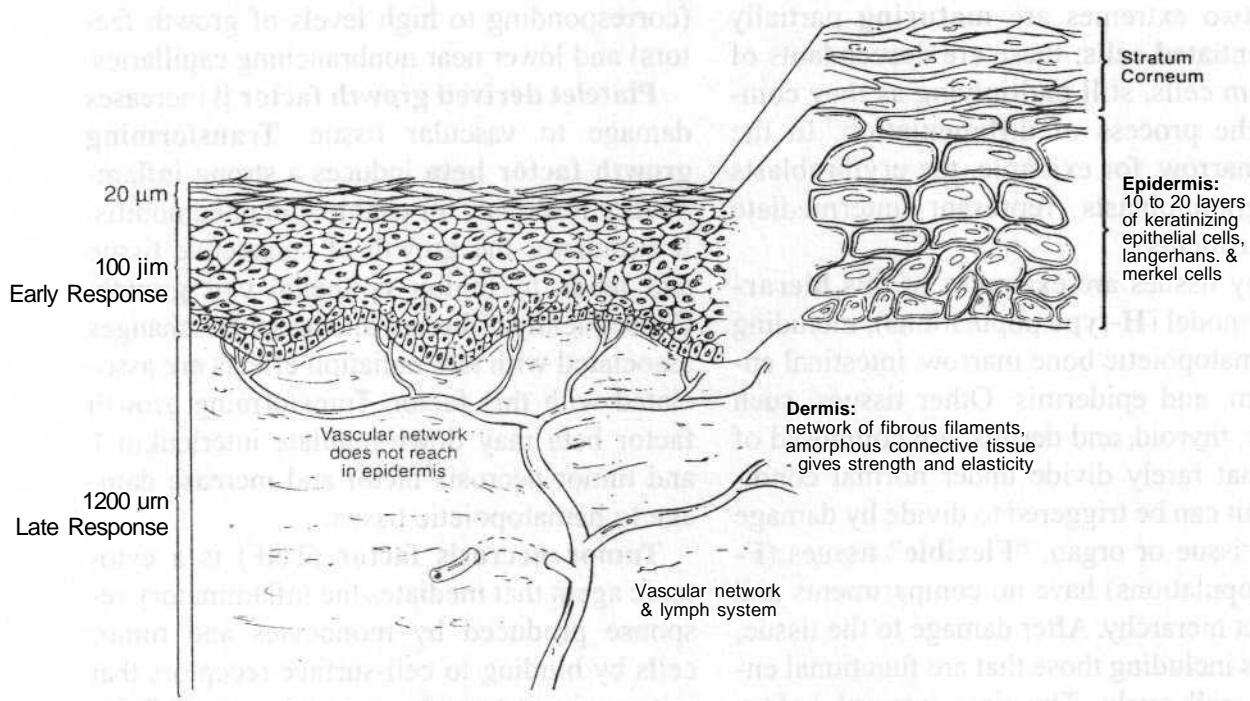
**Tumor necrosis factor** (TNF) is a cytotoxic agent that mediates the inflammatory response produced by monocytes and tumor cells by binding to cell-surface receptors that initiate signal-transduction pathways. TNF induces proliferation of fibroblasts, inflammatory cells, and endothelial cells and so is associated with complications. In clinical trials, the administration of TNF causes fatigue, anorexia, weight loss, and transient leukopenia. TNF protects hematopoietic cells and sensitizes tumor cells to radiation. Serum concentrations of TNF correlate with severity of pneumonitis, hepatic dysfunction, renal insufficiency, and demyelination; TNF may contribute to the pathophysiology of radiation-induced central nervous system symptoms. The expression of TNF following radiation is believed to be regulated at the transcriptional level and involves the protein kinase C-dependent pathway.

## SPECIFIC TISSUES AND ORGANS

### Skin

The skin is composed of the outer layer, the epidermis, which is the site of early radiation reactions, and the deeper layer, the dermis, which is the site of late radiation reactions (Fig. 19.2).

The epidermis (30-300  $\mu$ m thick) is derived from a basal layer of actively proliferating cells, which is covered by several layers of nondividing differentiating cells to the sur-



**Figure 19.2.** The skin from the perspective of a radiation biologist. The epidermis has a thickness of about 100  $\mu\text{m}$ , though it varies with body site (30-300  $\mu\text{m}$ ). It consists of 10 to 20 layers of keratinizing epithelial cells. This is a self-renewing tissue. The stem cell compartment forms part of the basal layer and has an unlimited capacity for proliferation. Cells produced in the basal layer migrate to the surface, differentiating as they do so, but retaining some proliferative potential. Cells in the surface layer are fully differentiated and keratinized and gradually are sloughed off and lost. The transit time for an epidermal cell to pass from the basal layer to the surface is 12 to 48 days, depending on the skin thickness. The dermis is about 1,200  $\mu\text{m}$  thick (1,000-3,000  $\mu\text{m}$ ) and consists of a dense network of fibrous fragments and connective tissue. The vascular network, capillaries, and lymph system are in the dermis. The vascular network does not extend into the epidermis. Two distinct waves of reactions are observed in the skin following irradiation. An early or acute reaction is observed about 10 days after a single dose and results from damage to the epidermis. Late reactions occur months later, mediated through damage to the dermis, principally to the vasculature. In clinical radiation therapy, late damage is now the dose-limiting reaction, because the build-up associated with megavoltage beams spares the epidermis.

face, at which the most superficial keratinized cells are desquamated. From the time a newly formed cell leaves the basal layer to the time it is desquamated from the surface is about 14 days. The target cells for radiation damage are the dividing stem cells in the basal layer.

The dermis is a dense connective tissue, 1 to 3 mm thick, within which scattered fibroblasts produce most of the dermal proteins. The vasculature of the dermis plays a major role in the radiation response. The target cells are thus the fibroblasts and the vascular endothelial cells.

A few hours after doses greater than 5 Gy (500 rad) there is an early erythema similar to

sunburn, which is caused by vasodilation, edema, and loss of plasma constituents from capillaries. Reactions resulting from stem cell death take longer to develop; when orthovoltage (250-kV) x-rays were the modality used commonly, skin was frequently dose-limiting, because the full dose is deposited in the superficial layers. In this case, an erythema develops in the second to third week of a fractionated regimen, followed by dry or moist desquamation resulting from depletion of the basal cell population. At lower doses, islets of skin may regrow from surviving stem cells (see the model scoring system in mouse skin developed and described by Withers, described in Chapter

18); at higher doses, at which there are no surviving stem cells within the area treated, moist desquamation is complete, and healing must occur by migration of cells from outside the treated area. With megavoltage x-ray equipment, the maximum dose ( $D_{max}$ ) occurs at a depth below the skin surface, from several millimeters to several centimeters, depending on the energy. Consequently, epidermal reactions usually are limited to dry desquamation and increased pigmentation. Conventional doses of 60 Gy (6,000 rad) or more are tolerated by the skin readily if they are spread out over 6 to 8 weeks, because a substantial amount of stem cell proliferation can occur during this time. For skin, as for oral mucosa, the total dose tolerated depends more on overall time than on fraction size.

With high energy x-rays,  $D_{max}$  occurs at a depth below the surface, so that late damage may occur in the dermis in the absence of early reactions in the epidermis. The clinical appearance of radiation fibrosis results from atrophy leading to contraction of the irradiated area. Telangiectasia developing more than a year after irradiation reflects late-developing vascular injury.

### Skin Appendages are a Special Case

Within a few days after irradiation, the death of germinal cells results in hair dysplasia (*i.e.*, short, thin hair). The proportion of dysplastic hair is dose-dependent. Epilation occurs during the third week, and regrowth may occur after 1 to 3 months. Sebaceous glands are as sensitive as hair, but sweat glands are less radiosensitive. Regenerated skin may be dry and hairless. An objective measure of skin damage may be obtained by a determination of electrical conductivity, which is influenced by sweat production.

### The Hematopoietic System

Hematopoietic tissues are located primarily in the bone marrow, with 60% located in the pelvis and vertebrae, and the remainder in the ribs, skull, sternum, scapula, and proximal

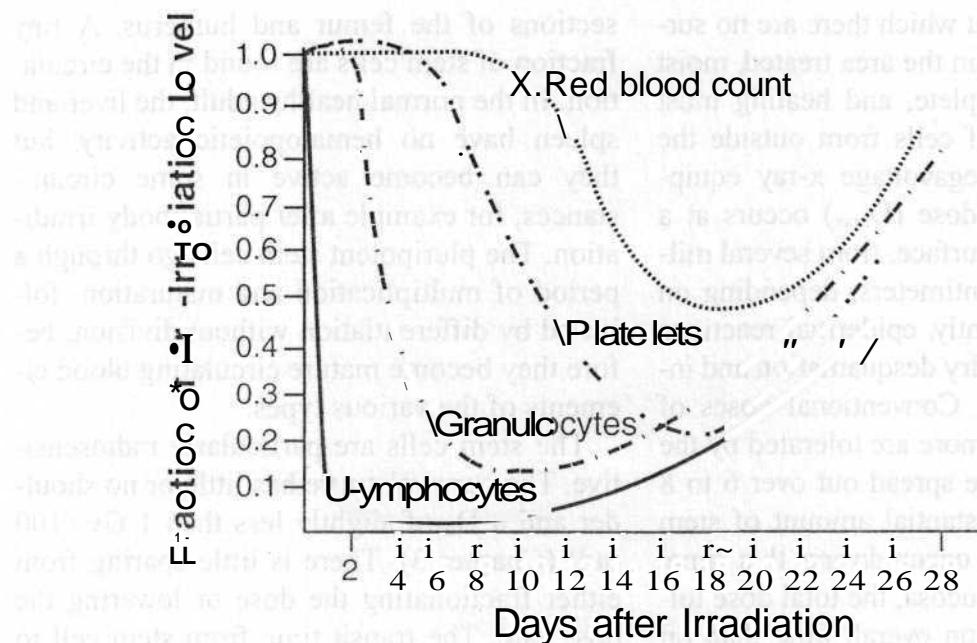
sections of the femur and humerus. A tiny fraction of stem cells are found in the circulation. In the normal healthy adult, the liver and spleen have no hematopoietic activity, but they can become active in some circumstances, for example after partial body irradiation. The pluripotent stem cells go through a period of multiplication and maturation, followed by differentiation without division, before they become mature circulating blood elements of the various types.

The stem cells are particularly radiosensitive. The survival curve has little or no shoulder and a  $D_0$  of slightly less than 1 Gy (100 rad) (Chapter 3). There is little sparing from either fractionating the dose or lowering the dose rate. The transit time from stem cell to fully functioning cell, however, differs for the various circulatory blood elements, and these differences account for the complex changes in blood count seen after irradiation.

### Blood Cell Counts after Total-body Irradiation

A dose as low as 0.3 Gy (30 rad) leads to a reduction in the number of lymphocytes, because they are among the most sensitive cells in the body. After larger doses, the number of all blood cells is altered; lymphopenia is followed by granulopenia, then thrombopenia, and finally anemia.

Following a total body dose of 4 to 6 Gy (400-600 rad), there is a temporary increase in the number of granulocytes, because of the mobilization of the reserve pool, followed by a rapid fall by the end of the first week. The number then remains almost constant, before falling again to a minimum value at 18 to 20 days after irradiation. After a week of aplasia, regeneration is rapid and takes place more or less simultaneously in platelets, **reticulocytes**, and granulocytes. After higher doses, the cell minimum is reached earlier and the period of aplasia lasts longer, increasing the possibility of hemorrhage and/or infection, which could prove fatal. At lower doses, around 1 Gy (100 rad), the depression in granulocyte count is less marked and regeneration less rapid. The



**Figure 19.3.** The pattern of depletion and recovery of the principal circulating elements of the blood following an intermediate dose of total-body radiation. The curves are purely illustrative: The time at which the nadir occurs is a combination of the radiosensitivity of the stem cells and the lifetime of the mature functional cells.

general pattern of the blood counts after a modest dose of radiation is illustrated in Figure 19.3.

The survival of stem cells determines the subsequent performance of the bone marrow after total-body irradiation, because in the first few hours there is a sudden decrease in the number of pluripotent stem cells and progenitor cells. If the number of stem cells falls below a critical level, production of functional cells essentially stops until partial regeneration of the stem cell compartment occurs and differentiation is allowed to resume. Administration of hematopoietic growth factors can shorten the period of aplasia markedly and accelerate regeneration of all blood cells.

#### *Partial-body Irradiation*

In the irradiated volume, the effects of partial-body irradiation are analogous to those following total-body irradiation. In the unirradiated marrow the stem cells start dividing within a few hours, and a compensatory hy-

perplasia attempts to maintain the total production of blood elements. There also may be an extension of hemopoiesis into the long bones, spleen, and liver, which are not normally hemopoietic in the adult human.

With fractionated radiation therapy, the pool of stem cells in the unirradiated volume falls progressively as differentiation is accelerated in an attempt to maintain the circulating blood count. Doses greater than about 30 Gy (3,000 rad) may cause permanent aplasia in the irradiated volume; hyperplasia and extension of the active bone marrow in the unirradiated volume may persist indefinitely.

Irradiation always reduces the number of stem cells in the bone marrow, and the return to normal may take a long time. This explains why patients remain sensitive to a new insult for months, or even years, following irradiation.

#### *Radiation and Chemotherapy Agents*

Some cytotoxic drugs act essentially on only those cells in cycle, and they have little

effect on hematopoietic stem cells, because 90% of them are out of cycle unless the marrow is regenerating following a previous insult. This explains why these drugs show extra toxicity if administered shortly after radiotherapy. The marrow of patients irradiated to a large volume is always more sensitive to cytotoxic drugs, partly because the pool of stem cells is reduced and partly because a greater proportion of stem cells is dividing actively.

### Lymphoid Tissue and the Immune System

The immune system is composed of macrophages and lymphocytes. Macrophages are derived from the same progenitors as granulocytes. These give rise to monocytes, which are transformed into macrophages. This cell line is less radiosensitive than lymphocytes, which are derived, however, from the same pluripotent stem cells.

The **p** line gives rise to (3 lymphocytes and plasmocytes, which are responsible for humoral immunologic responses and have life spans of 7 weeks and 2 to 3 days, respectively. Cells of the **T** line pass through the thymus, where they mature to become T lymphocytes. These cells have a lifespan of about 5 months and are responsible for cellular immunity and for secreting lymphokines. There are also other types of lymphocytes, including killer cells, responsible for antibody-dependent cytotoxic reactions, and natural killer cells, the function of which is not fully understood.

Total-body irradiation leads to a rapid fall in the number of circulating B and T lymphocytes, with the number returning to normal in a few weeks, depending on the dose. The lymphoid tissues (nodes, spleen, etc.) are very radiosensitive and are depleted of cells by quite small radiation doses. Lymphocytes are very radiosensitive, largely because of apoptosis; B cells are more radiosensitive than T cells and, overall, their radiosensitivity, measured by a clonogenic assay, is similar to hematopoietic stem cells.

The effect of irradiation on immune function is complex, depending on the volume ir-

radiated and the number of surviving cells, as well as their capacity to migrate and become lodged in the microenvironment. Total-body radiation is used to inhibit the immune system in preparing patients for an organ transplant, such as kidney or bone marrow. A total body dose of 3.5 to 4.5 Gy (350<sup>50</sup> rad) inhibits the immune response against a new antigen, though it is much less effective against an antigen to which the individual is already sensitized. The graft-versus-host reaction after bone marrow transplantation is relatively radioresistant. Partial-body irradiation, characteristic of ordinary radiation therapy, has only a limited effect on the immune response, and whether it influences metastatic dissemination is controversial. Total lymphoid irradiation to a dose of 30 to 40 Gy (3,000-4,000 rad) is used for the treatment of lymphomas and leads to a longlasting T-cell lymphopenia. It can be used to treat autoimmune diseases and also to prepare patients for organ transplants.

## The Digestive Tract

### Oral Mucosa

The cellular organization of the mucosa is similar to that of the skin, in that cells multiply in the basal layer and then migrate towards the surface as they differentiate. The lifespan of the differentiated cells, however, is much shorter than in the epidermis so that there is a more rapid reaction to radiation. The intensity of early mucous membrane reactions is a major factor limiting daily and weekly dose accumulation in the treatment of cancer of the head and neck (Table 19.2). For example, a schedule of 70 Gy (7,000 rad) delivered in 2-Gy (200-rad) fractions over 7 weeks leads to spotted-confluent mucositis in most patients, which approaches maximum tolerance if the schedule is accelerated to 5.5 weeks by, for example, the use of a concomitant boost technique.

The oral cavity contains a variety of tissue types, as well as the mucous membrane. The tongue consists of muscle bundles as well as mucosa with taste buds. The muscles undergo

TABLE 19.2. A Compilation of Tissue and Organ Sensitivities

	Injury	TD <sub>5/6</sub> , Gy	TD50/5 Gy	Field Size
<b>Class I organs</b>				
Bone marrow	Aplasia, pancytopenia	2.5	4.5	Whole segment
Liver	Acute and chronic hepatitis	30	40	Whole
		50	55	1/3
Intestine	Obstruction, perforation, fistula	40	55	Whole
		50	65	1/3 or 1/2
Stomach	Perforation, ulcer, hemorrhage	50	65	Whole
		60	70	1/3
Brain	Infarction, necrosis	45	60	Whole
		60	75	1/3
Spinal cord	Infarction, necrosis	47	—	20 cm
		50	70	5 or 10 cm
Heart	Pericarditis and pancarditis	40	50	Whole
		60	70	1/3
Lung	Acute and chronic pneumonitis	17.5	24.5	Whole
		45	65	1/3
Kidney	Acute and chronic nephrosclerosis	23	28	Whole
		50	45	1/3 or 1/2
<b>Class II organs</b>				
Oral cavity and pharynx	Ulceration, mucositis	60	75	50 cm <sup>2</sup>
Skin	Acute and chronic dermatitis, telangiectasia	55	65	100 cm <sup>2</sup>
Esophagus	Esophagitis, ulceration	55	50	Whole
		60	70	1/3
Rectum	Ulcer, stenosis, fistula	60	80	no vol effect
Salivary glands	Xerostomia	32	46	1/3 or 1/2
Bladder	Contracture	65	80	2/3
		80	85	1/3
Ureters	Stricture	70	100	5-10 cm length
Testes	Sterilization	1	2	Whole
Ovaries	Sterilization	2-3	6-12	Whole (age dep.)
Growing cartilage, child bone	Growth arrest, dwarfing	10	30	Whole
Mature cartilage, adult bone	Necrosis, fracture, sclerosis	60	100	Whole
		60	100	10 cm <sup>2</sup>
Eye				
Retina	Blindness	45	65	Whole
Cornea		50	6	Whole
Lens	Cataract	10	18	Whole
Endocrine				
Thyroid	Hypothyroidism	45	150	Whole
Adrenal	Hypoadrenalinism	60	—	Whole
Pituitary	Hypopituitarism	45	200	Whole
Peripheral nerves	Neuritis	60	100	—
Ear				
Middle	Serous otitis	30	40	No vol effect
Vestibular	Meniere's syndrome	60	70	—
<b>Class III organs</b>				
Muscle				
Child	Atrophy	20	40	Whole
Adult	Fibrosis	60	80	Whole
Lymph nodes and lymphatics	Atrophy, sclerosis	50	70	Whole node
Large arteries and veins	Sclerosis	80	100	10 cm <sup>2</sup>
Articular cartilage	None	500	5,000	Whole
Uterus	Necrosis, perforation	100	200	Whole
Vagina	Ulcer, fistula	90	100	Whole
Breast				
Child	No development	10	15	Whole
Adult	Atrophy, necrosis	50	100	Whole

Based on a combination of Rubin P, Casarett GW: Clinical Radiation Pathology, vol 1. Philadelphia, WB Saunders, 1968; and Emami et al., 1991, with permission.

Table compiled by Dr. Richard Miller. The figures in this table are a guide only.

mild progressive fibrosis and fiber atrophy after irradiation. The tonsils are lymphatic tissues and function as sites of antigen process and recognition. Following radiation exposure to a maximum tolerance dose, desquamation of the oral cavity occurs by about day 12, with recovery in 2 to 3 weeks. Desquamation occurs first in the soft palate, followed by the hypopharynx, vallecula, floor of the mouth, cheeks, epiglottis, base of tongue, vocal chords, and dorsum of tongue.

The sequence of events that occurs during radiation therapy for head and neck cancer is so important for the comfort and welfare of the patient that it is worth spelling out in detail. The order of events reflects the different kinetics of the cell populations involved:

**1st week:** Asymptomatic to slight focal hyperemia and edema caused by dilatation of capillaries in sensitive patients. Sensitivity may be associated with alcoholism or tobacco use, chemotherapy, infection (oral candidiasis, herpes simplex virus), immunosuppression (HIV).

**2nd week:** Increasing pain and loss of desire to eat. Sense of taste is altered; bitter and acid flavors are most changed, with less change with salty and sweet tastes. Erythema and edema increase, and early desquamative mucositis occurs. Basal cell division has been affected; this layer is being denuded, and vasculoconnective tissue damage becomes apparent. Mucositis is patchy.

**3rd week:** Mucositis and swelling with depletion of gland secretions leading to difficulty in swallowing. Mucositis plaques are confluent.

**4th week:** Progression of signs. Confluent mucositis sloughs, resulting in denuded lamina propria. Mucosa becomes covered by fibrin and polymorphonuclear leukocytes.

**5th week:** Maximum radiation damage apparent by this time. Extreme sensitivity to touch, temperature, and grainy food. Recovery of epithelial layer may begin during therapy.

*Posttherapy*, the basal cells migrate into the area and proliferate. In 2 to 4 weeks, complete resolution is observed. The serous acinar cells of the parotid and submaxillary salivary glands undergo interphase death, and hence salivary dysfunction appears after irradiation, with no threshold dose and little sparing by fractionation. Xerostomia is the main clinical effect that can interfere with nutrition, deteriorate oral hygiene, and predispose a patient to dental problems. TD<sub>5/5</sub> (the tolerance dose for 5% complication in 5 years) is 32 Gy (3,200 rad), and TD<sub>50/5</sub> (the tolerance dose for 50% complications in 5 years) is 46 Gy (4,600 rad). Impairment of taste acuity occurs during the third week of a multifraction radiotherapy regime.

### *Esophagus*

The mucosa consists of rapidly dividing cells. After radiation the esophagus displays an acute mucosal response of esophagitis and increased thickness of the squamous layer. Symptoms appear that include substernal burning with pain on swallowing at about 10 to 12 days after the start of therapy, with a return to normal within a week of the end of therapy. Late effects are related to the muscle layer; they include necrosis and a thickening of the epithelium. This leads to symptoms of difficulty on swallowing and possibly ulcerations after high doses. The tolerance dose is 57.5 Gy (5,750 rad) in 10 fractions (acute effects limited).

### *Stomach*

Irradiation of the stomach often causes nausea and vomiting immediately afterwards. The precursor cells of the gastric glands give rise to mucin-secreting surface columnar cells with short lifespans (about 3 or 4 days) and to acid-secreting parietal and pepsinogen-secreting chief cells that have long lifespans (hundreds of days). The precursor cells are radiosensitive, and their death leads to early depletion of the surface columnar cell epithelium. Delayed gastric emptying and epithelial denudement are the two main early radiation

effects. Peptic ulceration is seen in patients receiving more than 40 Gy (4,000 rad) to the upper abdomen.

Dyspepsia may be evident in 6 months to 4 years and gastritis in 1 to 12 months. Acute ulceration may occur shortly after the completion of treatment but rarely leads to perforation. At about 5 months, late ulceration and submucosal fibrosis leading to antral fibrosis may occur. Tolerance doses range from 40 to 50 Gy (4,000-5,000 rad).

### ***Small and Large Intestine***

As with the skin and oral mucosa, both early and late complications are observed in the gastrointestinal tract. Acute mucositis frequently occurs, with symptoms such as diarrhea or gastritis, depending on the treatment field. If the dose is limited to 50 to 54 Gy (5,000-5,400 rad) in 2-Gy (200-rad) fractions, acute reactions are seldom dose-limiting, and if they do occur a few days' interruption of treatment usually alleviates the problem. Much more serious are the long-term late sequelae, which may develop either from persistent severe early reactions (consequential late effects) or independently of acute damage in the submucosal, muscular, or serosal layers.

In the small intestine, stem cells are located toward the bottom of the crypts of Lieberkuhn. Atrophy of the villus occurs about 2 to 4 days postirradiation. Epithelial denudation is responsible for the acute gut reactions. A regenerative response appears rapidly, and within 2 to 4 days micro- and macrocolonies are detectable. The surviving crypts have, at least, the same radiosensitivity to reirradiation as the unirradiated crypts, and very little dose is "remembered."

Late bowel reactions involve all tissue layers and are caused by atrophy of the mucosa caused by vascular injury, with subsequent breakdown resulting from mechanical irritation and bacterial infection, which leads to an acute inflammatory response. Also, overgrowth of the fibromuscular tissue with stenosis and serosal breakdown and adhesion formation may occur, which may be predis-

posed to by previous surgery and is related to inflammatory mediators. Fibrosis and ischemia are typical late effects.

Tolerance dose is about 50 Gy (5,000 rad) for the small intestine and slightly higher for the large intestine. Rectal tolerance is about 70 Gy (7,000 rad).

### **Lung**

The lung is an intermediate- to late-responding tissue. Two waves of damage can be identified: acute pneumonitis at 2 to 6 months after treatment, and fibrosis, which may develop slowly over a period of several months to years. The only symptom of early acute pneumonopathy may be an opacity on a chest x-ray, though it may be accompanied by functional signs including cough, dyspnea, and respiratory difficulties. Progressive pulmonary fibrosis develops in most patients, including those who previously were asymptomatic, beginning about a year after irradiation.

Difficulties in respiratory function increase in severity with time and are generally irreversible. Their severity depends on three factors; volume irradiated, dose, and fraction size. The lung is particularly sensitive to fractionation, with an  $\alpha/\beta$  estimated to be about 3 Gy (300 rad). The most likely target cells are the pulmonary endothelial cells and the type II pneumocytes (cells of the alveolar wall). Type II cells are associated with the production of surfactant during the first few days after irradiation.

The lung is among the most sensitive of late-responding organs. The FSU in the lung is the pulmonary lobule, consisting of the terminal bronchioli and respiratory parenchyma that it serves. The FSUs are arranged in parallel, in that a large number of bronchi and alveoli work together; consequently, volume as well as dose is important. Because of this organization of the functional units, the lung is only dose-limiting if large volumes are irradiated, and if the remaining lung is not capable of providing adequate function.

Pulmonary damage also may occur following use of chemotherapy agents, notably

bleomycin, cyclophosphamide, and mustine. Combination of radiation with these drugs reduces lung tolerance.

### Kidney

Together with the lung, the kidney is among the more radiosensitive late-responding critical organs (Table 19.2). Irradiation of both kidneys to a modest dose of about 30 Gy in 2-Gy fractions (3,000 rad in 200-rad fractions) results in nephropathy with arterial hypertension and anemia. Radiation damage develops slowly and may not become evident for years. Parts of one or even both kidneys can receive much higher doses. In contrast to most organs or tissues, increasing treatment time does not allow higher doses to be tolerated. Functional units are arranged in parallel, with each containing only about 1,000 stem cells. Damage to tubules, therefore, may result from sterilization of all the cells in a tubule.

### Liver

In terms of radiosensitivity, the liver ranks immediately below kidney and lung. It shares with these organs the fact that its functional subunits are arranged in parallel, so that much larger doses are tolerated if only part of the organ is exposed.

Liver tolerance is **dose-limiting** only if the whole organ is irradiated, as in, for example, total-body irradiation prior to bone-marrow transplantation. The lifespan of a hepatocyte is about 1 year, so that under normal conditions the cell-renewal rate in the liver is very slow. Even large doses apparently are tolerated for a few months, but then hepatic function deteriorates progressively. Fatal hepatitis may result from a fractionated protocol of only 35 Gy (3,500 rad) if the whole organ is irradiated.

### Bladder Epithelium

The epithelium of the bladder consists of a basal layer formed of small diploid cells, cov-

ered by several layers of larger transitional cells and at the surface by a layer of very large polyploid cells with a thick membrane designed to resist the irritation caused by urine. Cell-renewal rate is low, the superficial cells having a lifespan of several months. Because of this long lifespan, accelerated proliferation following irradiation does not begin for months. Senescence of the differentiated functional cells then reveals latent damage in the basal layer. Frequency of urination increases in parallel with bladder damage and loss of surface cells. The absence of these surface cells explains the irritation by urine of the deeper cellular layers, leading to stimulation of cellular proliferation. Subsequent late effects are related to fibrosis and reduction in bladder capacity.

### Central and Peripheral Nervous System

The nervous system is less sensitive to radiation than other late-responding organs and tissues such as the kidney or lung. Although tolerance doses are frequently quoted at the 5% complication level (*i.e.*, **TBs**), wide margins of safety in dose usually are included, because damage to these tissues result in severe consequences, including paralysis.

#### Brain

Three main categories of cells are involved: neurons, vascular endothelial cells, and glial cells. Neurons are nonproliferating end-cells in adults; glial cells have a slow rate of turnover, with a small precursor (stem cell) compartment of only about 1%. Endothelial cells also have a slow turnover but can proliferate rapidly after injury. The most important injuries to the brain by radiation are all late syndromes, developing months to years after exposure. Some reactions occur within the first 6 months, including transient demyelination (somnolence syndrome) or the much more serious leukoencephalopathy. Typical radiation necrosis may become evident as early as 6 months but may be delayed as long as 2 to 3 years. Histopathologic

changes that occur within the first year are most likely to involve white matter, whereas for times beyond 6 to 12 months the grey matter usually shows changes accompanied by vascular lesions such as telangiectasia and focal hemorrhages. A mixture of histologic characteristics is likely to be associated with radionecrosis manifest from 1 to 2 years postirradiation; accompanied by cognitive defects.

### Spinal Cord

Radiation-induced changes in the spinal cord are similar to those seen in the brain as far as latency, tolerance dose, and histology are concerned. Lhermitte's sign is a demyelinating injury that develops early, by several months after treatment, persists for a few months to a year, but is usually reversible. It may occur at doses as low as 35 Gy (3,500 rad), well below the tolerance dose for permanent radiation myelopathy, and its appearance does not predict later more serious problems.

Late damage includes two principal syndromes. The first, occurring from about 6 to 18 months, involves demyelination and necrosis of the white matter; the second is mostly a vasculopathy and has a latency of 1 to 4 years.

For the spinal cord, the **TD<sub>5/5</sub>** is about 50 Gy (5,000 rad) for a 10-cm length irradiated, and 55 Gy (5,500 rad) for a **5-cm** length. By 70 Gy (7,000 rad) the incidence of myelopathy would be about 50%.

The tolerance dose to the spine shows little dependence on overall treatment time, at least for protocols of conventional length up to 10 weeks. By contrast, tolerance depends critically on dose per fraction. Lower doses per fraction reduce the risk of late effects, but if two doses per day are used, the time between fractions must be at least 6 hours (and preferably more), because the repair of sublethal damage is slow in this tissue. There is evidence of two components of repair, one with a half-time less than 1 hour and one with a half-time close to 4 hours. The spinal cord is the clearest example of a tissue in

which FSUs are arranged in series. The probability of a myelopathy depends critically on the length irradiated for very small lengths, but once the length of the field exceeds a few centimeters, the treatment volume has little effect.

Caution must be exercised in combining radiation with chemotherapy agents, because neurotoxic agents such as methotrexate, *cis*-platinum, vinblastine, and Ara C reduce the tolerance to radiation delivered simultaneously or sequentially.

As far as re-treatment is concerned, animal data suggest that by about two years, the majority of the damage from a prior exposure has been repaired; the extent of the repair depends very much on the level of the initial injury.

### Peripheral Nerves

Radiation injury of peripheral nerves probably is more common than effects on the spinal cord. It is often said that peripheral nerves are more radioresistant than the cord or brain, but there are few quantitative data to support this. Sixty grays (6,000 rad) in a conventional regimen of 2-Gy (200-rad) fractions may lead to a 5% probability of injury, with the probability rising steeply thereafter with increasing dose.

### The Testes

The seminiferous tubules are composed of two types of cells: sertoli cells, which secrete a hormone which controls the secretion of FSH by the hypophysis; and the germinal cells, the hierarchy of which is strictly defined. The stem cells, the type A spermatogonia, have a long cell cycle and divide infrequently. The process of differentiation proceeds through several types of spermatogonia to the spermatocytes, which are the cells in which meiosis occurs. Each spermatocyte gives rise to four spermatids, which finally result in spermatozoa. In humans, the transit time from stem cell to spermatozoa is about 74 days. There is considerable cell loss

along the way, so that the amplification factor is much less than might be calculated from the number of divisions that occur.

Leydig cells, which secrete testosterone, also are found in the testis, and their function is regulated by pituitary gonadotrophins, prolactin, and luteinizing hormone. This is important in the use of neoadjuvant hormone therapy during the treatment of prostatic cancer.

In humans, a dose as low as 0.1 Gy (10 rad) leads to a temporary reduction in the number of spermatozoa, and 0.15 Gy (15 rad) leads to temporary sterility. Azoospermia lasting for several years occurs after 2 Gy (200 rad), and permanent azoospermia after about 6 to 8 Gy (600-800 rad). On the other hand, even much larger doses have little effect on the Leydig cells in the adult, so that although irradiation of the testes may lead to sterility, it has little or no effect on the libido.

The stem cells appear to be more radiosensitive than the differentiating spermatogonia, which explains why the duration of azoospermia increases as the dose is increased. Fractionated or continuous low dose rate irradiation is more effective than a single acute exposure, because a large proportion of the stem cells are in a radioresistant phase of the cell cycle. If irradiation is protracted, it affects stem cells as they move through the cell cycle into more radiosensitive phases. This accounts for the longlasting azoospermia seen after relatively low daily doses of scattered radiation reaching the testes during irradiation of the pelvis, and also the occurrence of testicular dysfunction seen after years of occupational exposure to ionizing radiation.

A number of cytotoxic drugs have substantial effects on spermatogenesis. For example, the alkylating agents included in MOPP (mechlorethamine, vincristine, procarbazine, and prednisone), the combination of chemotherapy agents used at one time for the treatment of Hodgkin's lymphoma, led to sterility in almost all patients. Of course, the drug treatment was prolonged and simulated low dose rate irradiation, killing stem cells as they came into cycle.

### The Ovaries

The effects of radiation on the ovaries are quite different from those on the testes because, after the fetal stage, the oocytes no longer divide. They are all present at birth, and their number diminishes steadily with age, reaching zero by the time of menopause. Oocytes are extremely radiosensitive to cell killing; like lymphocytes they die in interphase death, with  $D_0$  of only 0.12 Gy (12 rad). There is little effect of fractionation. Mature follicles and those in the process of maturation are damaged equally by radiation, so that sterilization is immediate (*i.e.*, no latent period, as in the male). Because hormonal secretion is associated with follicular maturation, sterilization by radiation leads to a loss of libido and all of the changes associated with menopause.

### The Female Genitalia

The skin of the vulva reacts like skin elsewhere, but because of moisture and friction, a tolerance dose of 50 to 70 Gy (5,000-7,000 rad) in conventional fractions is considered to be on the high side.

Acute effects of irradiation of the vagina include erythema, moist desquamation, and confluent mucositis, leading to the loss of vaginal epithelium that may persist for 3 to 6 months. Gross abnormalities in the vagina may include pale color, a thin atrophic nucosa, inflammation, and tissue necrosis with ulceration leading to a fistula. Tolerance doses, however, are high; 90 Gy (9,000 rad) before ulceration and 100 Gy (10,000 rad) for the development of a fistula. From intracavitary treatments, doses to the cervix and uterus may reach as high as 200 Gy (20,000 rad). Effects seen include atrophy of the endometrial glands and stroma, as well as ulceration.

### Blood Vessels and The Vascular System

The effects of radiation on blood vessels is particularly important, because late damage

to many different tissues and organs is mediated to some extent by effects on the vasculature. Blood vessels have a complex structure. A monolayer of endothelial cells lines the interior surface, resting on connective tissue, the thickness of which depends on the type of vessel. Under normal circumstances the rate of proliferation of endothelial cells is low so that, following exposure to radiation, cell loss occurs over a period of time as cells enter mitosis. Regions of constriction appear because of the abnormal proliferation of surviving cells. Denudation of the surface of blood vessels leads to the formation of thromboses and capillary necroses. In the smooth muscle cells that make up the wall of blood vessels, the proportion of cells cycling is very low, so that it takes several years for the number of cells to diminish significantly following irradiation. The loss of muscular fibers plays an important role in the development of late damage that may become evident several years later. Muscle cells are replaced by collagen fibers, vessel walls lose their elasticity, and blood flow is diminished.

Arterial damage may occur after doses of 50 to 70 Gy (5,000-7,000 rad), delivered in conventional fractionation patterns, but capillaries are damaged by doses above about 40 Gy (4,000 rad). In general, veins are less sensitive to radiation than arteries.

### The Heart

In its tolerance to radiation the heart is intermediate between the kidney or lung and the central nervous system. The most common radiation-induced heart injury is acute pericarditis, which seldom occurs during the first year posttherapy. It varies in severity from transient pericarditis, which runs a benign course, to dense sclerosis with cardiac constriction. Anterior chest pain with shortness of breath and low-grade fever may be observed. The threshold dose may be as low as 20 Gy (2,000 rad) if more than 50% of the heart is irradiated, but higher for partial exposure. A

dose of 45 to 50 Gy (4,500-5,000 rad) produces an 11% incidence. The *oc/p* ratio for the heart is low (about 1 Gy or 100 rad), so that fractionation results in a substantial sparing effect.

Radiation-induced cardiomyopathy results from dense and diffuse fibrosis; it is a slowly evolving lesion that develops over a period of many years and leads to impaired function. Reduced cardiac function is seen in some patients with Hodgkin's disease who receive a dose of about 30 Gy (3,000 rad) to most of the heart. Protection of part of the heart greatly reduces the incidence of symptoms.

The chemotherapy agent adriamycin increases the severity of radiation-induced complications. In addition, adriamycin may reveal latent radiation damage many years after radiation therapy.

### Bone and Cartilage

In children, growing cartilage is particularly radiosensitive. Doses as low as 10 Gy (1,000 rad) can slow growth because of the death of chondroblasts. Above about 20 Gy (2,000 rad), the deficit in growth is irreversible. The effects of radiation on bone growth are more serious for higher doses and for younger ages. Sequelae are particularly serious in children younger than 2 years of age, and radiation can affect stature adversely up to the time of puberty.

In the adult, osteonecrosis of the lower maxilla may be a serious complication following radiation therapy for cancer of the buccal cavity. The TD<sub>5/5</sub> is 50 to 60 Gy (5,000-6,000 rad); the TD<sub>50/5</sub> is about 70 Gy (7,000 rad) for large irradiated volumes. Fractures of the humeral and femoral head are observed if the dose, in conventional fractions, is high. The TD<sub>5</sub> is 52 Gy (5,200 rad), and the TD<sub>50/5</sub> is 65 Gy (6,500 rad). Fractures of the ribs and clavicle are sometimes seen in patients receiving radiotherapy for breast cancer but are generally not serious complications.

### SUMMARY OF PERTINENT CONCLUSIONS

- Apparent radioresponsiveness of a tissue depends on
  1. Inherent sensitivity of cells
  2. Kinetics of the cell population
- Sensitivity of actively dividing cells is expressed by their survival curve for reproductive integrity.
- The radiation dose needed to destroy the functioning ability of a differentiated cell *is far greater than* that necessary to stop the mitotic activity of a dividing cell.
- The shape of the dose-response relationship for functional endpoints, obtained from multifraction experiments, is more pertinent to radiotherapy than clonogenic assays.
- The time interval between irradiation and its expression in tissue damage depends on the lifespan of mature functional cells and the time it takes for a cell born in the stem compartment to mature.
- Hyperthermia damage is expressed early compared with radiation damage.
- Both early and late effects may develop in one organ system, because of injury to different target cell populations or tissue elements.
- The ratio  $a/3$  (the dose at which the linear and quadratic components of radiation damage are equal) may be inferred from multifraction experiments in systems scoring non-clonogenic endpoints.
- Tolerance doses for late effects are more sensitive to changes in dose per fraction (low  $\alpha/\beta$  value) compared with early effects.
- Spatial arrangement of functional subunits (FSUs) is critical to the tolerance of some normal tissues.
- In some tissues (*e.g.*, spinal cord), the FSUs are arranged serially (like links in a chain), and the integrity of each is critical to organ function.
- Tolerance depends more critically on volume irradiated for tissues in which FSUs are arranged serially.
- A tissue with intrinsically high tolerance may fail as a result of the inactivation of a small segment (as in the spinal cord); a tissue with an intrinsically low tolerance (kidney, lung) may lose a substantial number of its functional units without impact on clinical tolerance.
- Casarett's classification of tissue radiosensitivity is based on histopathologic observations.
- In terms of radiosensitivity based on histologic observation of cell death, parenchymal cells fall into four categories, from most sensitive to most resistant:
  - I. Stem cells of classic self-renewal tissues, which divide regularly
  - II. Differentiating intermitotic cells, which divide regularly but in which there is some differentiation between divisions
  - III. Reverting postmitotic cells, which do not divide regularly but can divide under the appropriate stimulus
  - IV Fixed postmitotic cells, which are highly differentiated and appear to have lost the ability to divide
- Connective tissue and blood vessels are intermediate in radiosensitivity between groups II and III. There is a longstanding dispute as to whether the late effects of radiotherapy are caused principally by damage to parenchymal cells or damage to the vasculature.
- Michalowski's classification divides tissues into hierarchical (Fl-type) and flexible (F-type) populations, which respond differently to radiation.

- Many tissues are a hybrid of H-type and F-type.
- The response of a tissue is influenced greatly by a host of growth factors, including interleukins 1 and 6, basic fibroblast growth factor, platelet derived growth factor (3), transforming growth factor (3), and tumor necrosis factor.
- Early radiation response in the skin is caused by damage to the epidermis; the late response reflects damage to the epidermis.
- The hematopoietic system is very sensitive to radiation, especially the stem cells. The complex changes seen in peripheral blood count after irradiation reflect differences in transit time from stem cell to functioning cell for the various circulatory blood elements.
- The effect of irradiation on the immune function is complex, depending on the volume irradiated and the number of surviving cells. A total-body dose of 3.5 to 4.5 Gy (350-450 rad) inhibits the immune response against a new antigen.
- The cellular organization of the lining of the gastrointestinal tract is similar to the skin, but the life span of the differentiated cells is shorter. Both early and late sequelae can occur.

*Oral mucosa:* Damage to the oral mucosa during radiotherapy for head and neck cancer is very important for both the comfort and welfare of the patient. Xerostomia can interfere with nutrition and dental health.

*Esophagus:* Early and late effects can occur and lead to difficulty in swallowing. Tolerance is 57.5 Gy (5,750 rad) in 10 fractions (acute-effect limit).

*Stomach:* Irradiation of the stomach often leads to nausea and vomiting. Tolerance doses range from 40 to 50 Gy (4,000-5,000 rad).

*Small and large intestines:* Both early and late complications can occur. Tolerance dose is about 50 Gy (5,000 rad) for the small intestine, slightly higher for the large intestine, and 70 Gy (7,000 rad) for the rectum.

- The lung is an intermediate- to late-responding tissue. Two waves of damage can be identified, an acute pneumonitis and a later fibrosis. The lung is among the most sensitive late-responding organs. Pulmonary damage also may occur following chemotherapy.
- Together with the lung, the kidney is among the more radiosensitive late-responding critical organs. FSUs are in parallel, with only about 100 stem cells in each. Thirty grays (3,000 rad) in 2-Gy (200-rad) fractions to both kidneys results in nephropathy.
- In terms of radiosensitivity, the liver ranks immediately below kidney and lung. FSUs are in parallel, so that much larger doses are tolerated if only part of the organ is exposed. Fatal hepatitis may result from 35 Gy (3,500 rad) (conventional fractionation) to the whole organ.
- Cell renewal is low in the bladder epithelium, so proliferation following irradiation is delayed. Frequency of urination increases in parallel with loss of surface cells. Absence of surface cells explains irritation by urine.
- *Central and peripheral nervous system:* The nervous system is less sensitive to radiation than other late-responding organs such as the kidney or lung.

*Brain:* Histopathologic changes that occur in the first year are most likely to involve white matter; at later times, grey matter usually shows changes accompanied by vascular lesions. Radionecrosis may occur accompanied by cognitive defects.

*Spinal Cord:* Early demyelinating injuries may develop after doses as low as 35 Gy (3,500 rad) but are usually reversible. For late damage, the TD<sub>5/5</sub> is about 50 Gy (5,000 rad) for a 10-cm length of cord. By 70 Gy (7,000 rad) in conventional fractions, the incidence of myelopathy would be 50%. FSUs are in series, but once the field exceeds a few centimeters, the treatment volume has little effect. Tolerance dose shows little de-

pendence on overall time but depends critically on dose per fraction (oc/p is low). If two doses per day are used, the interfractionation interval must be more than 6 hours, because there is a slow component of repair.

- In the testes a dose of 0.1 to 0.15 Gy (10-15 rad) leads to temporary sterility. Six to eight grays (600-800 rad) in 2-Gy (200-rad) fractions leads to permanent sterility. Such doses have little effect on libido. The stem cells are more radiosensitive than the differentiated cells, so that continuous or fractionated radiation is more effective than a single acute dose.
- Sterilization by radiation to the ovaries is immediate (no latent period as in the male) and leads to all the changes associated with menopause.
- Among the female genitalia, tolerance doses for the vagina are high: 90 Gy (9,000 rad) before ulceration and 100 Gy (10,000 rad) for the development of a fistula. From intracavitary treatment, doses to the cervix and uterus may reach 200 Gy (20,000 rad).
- Late damage to many different tissues and organs is mediated to some extent by effects on the vasculature. Arterial damage may occur after fractionated doses of 50 to 70 Gy (5,000-7,000 rad), but capillaries are damaged by doses about 40 Gy (4,000 rad).
- In its tolerance to radiation, the heart is intermediate between the kidney or lung and the central nervous system. The most common radiation-induced heart injury is acute pericarditis, which seldom occurs in the first year posttherapy. Forty to fifty grays (4,500-5000 rad) in conventional fractions induces about an 11% incidence. The  $a/(3)$  is low (1 Gy or 100 rad) so that fractionation results in a substantial sparing. Protection of part of the heart reduces symptoms.
- Growing cartilage is particularly radiosensitive in children: 10 Gy (1,000 rad) can slow growth, and deficits in growth are irreversible at about 20 Gy (2,000 rad). In the adult, osteoporosis of the lower mandible may be a serious complication following radiotherapy for cancer of the buccal cavity. Fractures of the humeral or femoral head may occur; the TD<sub>50/5</sub> is about 65 Gy (6,500 rad).

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## Model Tumor Systems

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TRANSPLANTABLE SOLID TUMOR SYSTEMS IN EXPERIMENTAL ANIMALS  
TUMOR-GROWTH MEASUREMENTS  
TCD<sub>50</sub> ASSAY  
DILUTION ASSAY TECHNIQUE  
LUNG COLONY ASSAY  
IN VIVO IN VITRO TECHNIQUE  
XENOGRAFTS OF HUMAN TUMORS

SPHEROIDS: AN *IN VITRO* MODEL TUMOR SYSTEM  
SPHEROIDS OF HUMAN TUMOR CELLS  
COMPARISON OF THE VARIOUS MODEL TUMOR SYSTEMS  
APOPTOSIS IN TUMORS  
SUMMARY OF PERTINENT CONCLUSIONS

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### TRANSPLANTABLE SOLID TUMOR SYSTEMS IN EXPERIMENTAL ANIMALS

A wide range of experimental tumors of various histologic types have been developed for radiobiologic studies. To produce a large number of virtually identical tumors, propagation by transplantation from one generation of animals to the next is used, which makes it mandatory that the animals be isologous. In practice, pure inbred strains of rats or mice are used and are maintained by brother-sister mating, which also serves the function of reducing the variability among animals to a minimum.

The tumor from a donor animal is removed aseptically and if possible prepared into a single-cell suspension; this is accomplished by separating the cells with an enzyme such as trypsin and then forcing them through a fine wire mesh. To effect a transplant, 10<sup>4</sup> to 10<sup>6</sup> cells are inoculated subcutaneously into each of a large group of recipient animals of the same strain. The site of transplantation varies widely; the flank or back commonly is used,

but sometimes a special tumor requires a particular site, such as the brain. Some tumors cannot be handled in this way and must be propagated by transplanting a small piece of tumor rather than a known number of single cells; this is obviously less quantitative. Within days or weeks, depending on the type of tumor and the strain of animals, palpable tumors appear in the recipient animals that are uniform in size, histologic type, and so on. Hundreds to thousands of animals can be used, which makes it possible to design highly quantitative studies of tumor response to different radiations, fractionation regimens, sensitizers, and combinations of radiation and chemotherapeutic agents.

There are five commonly used techniques to assay the response of solid tumors to a treatment regimen:

1. Tumor growth measurements
2. Tumor cure (TCD<sub>50</sub>)
3. Tumor cell survival determined *in vivo* by the dilution assay technique
4. Tumor cell survival assayed by the lung colony system

5. Tumor cell survival—*in vivo* treatment followed by *in vitro* assay

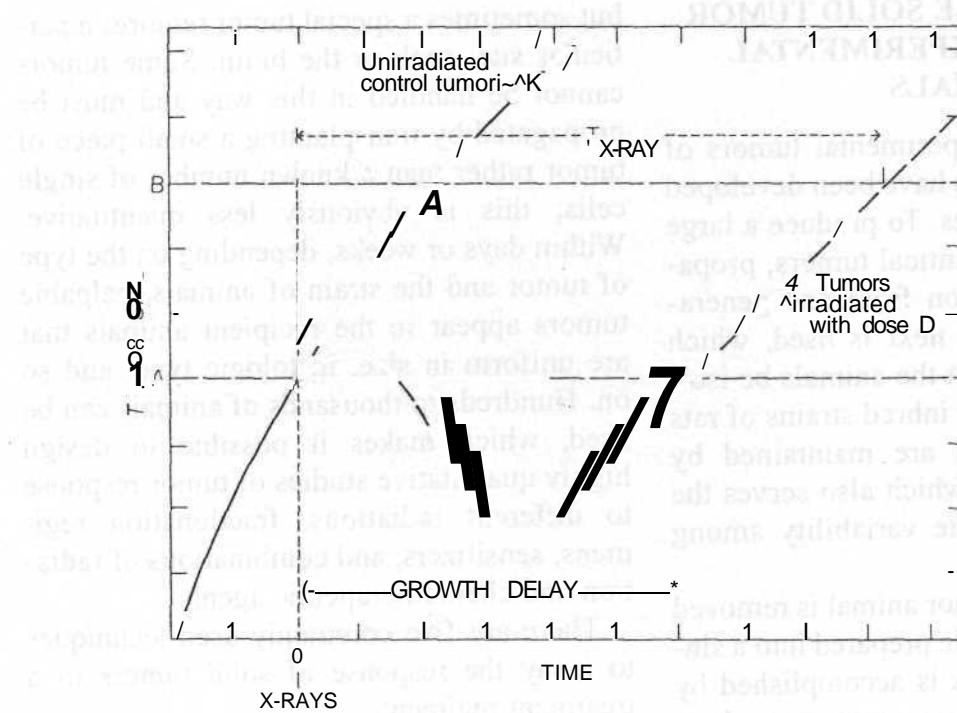
Each of these methods is discussed briefly.

### TUMOR-GROWTH MEASUREMENTS

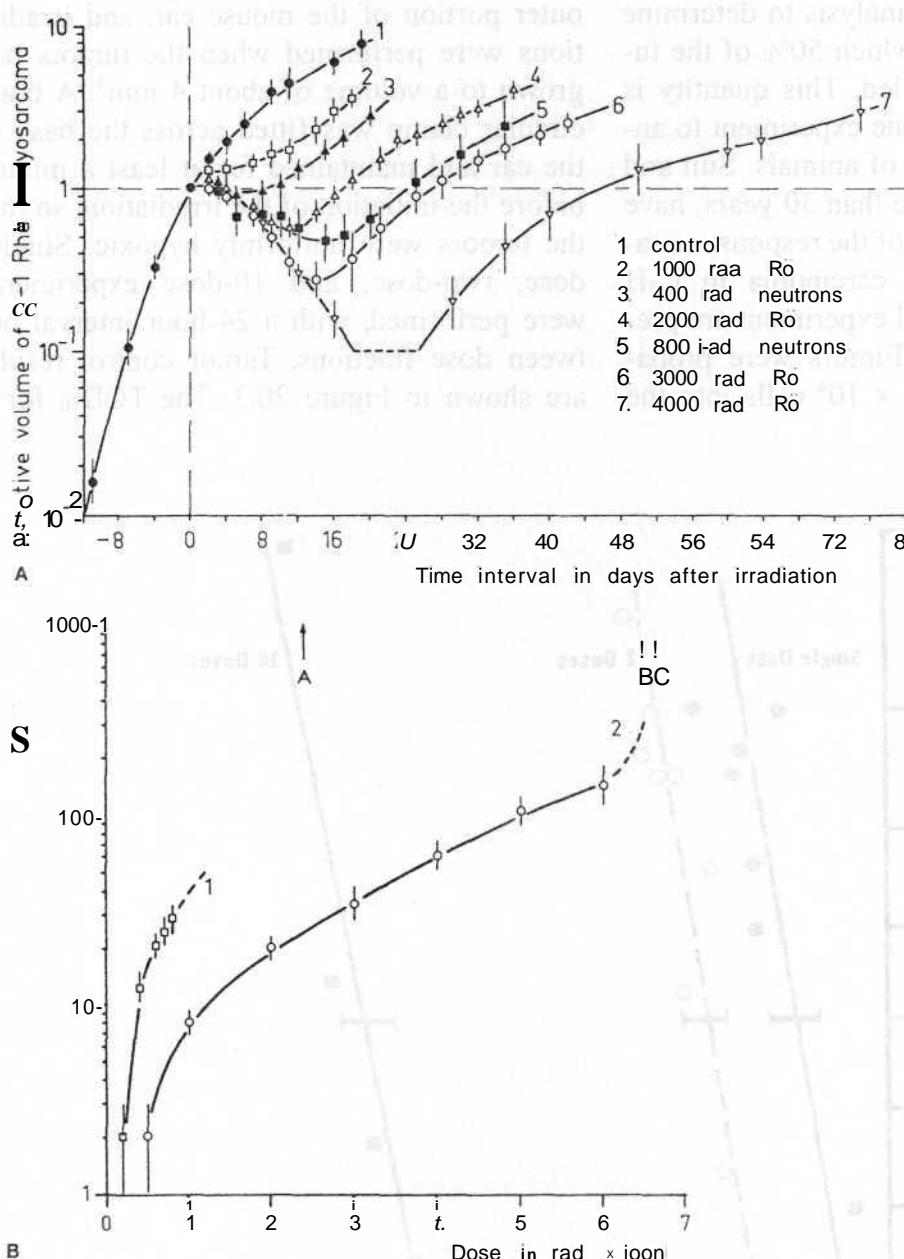
Tumor-growth measurement is possibly the simplest endpoint to use and involves the daily measurement of each tumor to arrive at a mean diameter. For tumor-growth experiments a large number of transplanted tumors are prepared as previously described. When they have grown to a specified size (e.g., a diameter of 8 to 10 mm in rats or 2 to 4 mm in mice), they are treated according to the plan of the particular experiment. Figure 20.1 illustrates the variation of tumor size with time for unirradiated controls and tumors given a single dose of x-rays. The untreated tumors grow rapidly at a relatively uniform rate; the

radiation treatment causes a temporary shrinkage of the tumor, followed by regrowth.

Two different methods have been used to score the tumor response. Barendsen and his colleagues have used growth delay, illustrated in Figure 20.1, as the time taken after irradiation for the tumor to regrow to the size it was at the time of irradiation. Clearly, this index of response is only suitable for tumors that shrink significantly after irradiation. For tumors that do not shrink so obviously, a more convenient measure of growth delay is the time taken for the irradiated tumor to grow to some specified size after exposure, compared with controls. Either index of growth delay increases as a function of dose. Figure 20.2A shows growth curves for a rat rhabdomyosarcoma irradiated with various doses of x-rays or fast neutrons. In Figure 20.2B, growth delay is expressed as a function of radiation dose.



**Figure 20.1.** The pattern of response of a tumor to a dose of x-rays. The size of the tumor, either the mean diameter or the volume, is plotted as a function of time after irradiation. Two different indices of tumor responses have been used by different investigators. Growth delay represents the time after irradiation that it takes for the tumor to regrow to the size at the time of irradiation. Alternatively, the index of radiation damage may be the time taken for the tumor to grow from a specified size A at the time of irradiation to some specified larger size B. Typically, this may be from 9 to 25 mm in diameter for rat tumors. This quantity is shown as TCON for unirradiated control animals and TX-RAY for tumors irradiated with a dose (D) of x-rays. Either index of tumor response may be plotted as a function of radiation dose.



**Figure 20.2. A:** Volume changes of rhabdomyosarcomas in rats after irradiation. Curve 1 represents the growth of the unirradiated control tumors. Curves 2, 4, 6, and 7 refer to tumors irradiated with 10 to 40 Gy (1,000-4,000 rad) of 300-kV x-rays. Curves 3 and 5 refer to tumors irradiated with 4 and 8 Gy (400 and 800 rad) of 15-MeV  $d_{\alpha} \rightarrow T$  fast neutrons. **B:** Growth delay of rhabdomyosarcomas in rats as a function of dose of x-rays (curve 2) or fast neutrons (curve 1). A and C indicate the doses of neutrons and x-rays required to "cure" 90% of the tumors, calculated on the basis of cell-survival curves. 6 indicates the observed TCD<sub>90</sub> for x-rays. Note the good agreement between calculated and observed values of the TCD<sub>90</sub> for x-rays. (From Barendsen GW, Broerse JJ: Experimental radiotherapy of a rat rhabdomyosarcoma with 15 MeV neutrons and 300 kV x-rays: I. Effects of single exposures. Eur J Cancer 5:373-391, 1969, with permission.)

#### TCD<sub>50</sub> ASSAY

Tumor control provides data of most obvious relevance to radiotherapy. In experiments of this kind a large number of animals with tumors of uniform size is divided into separate

groups, and the tumors are irradiated locally with graded doses. The tumors subsequently are observed regularly for recurrence or local control. The proportion of tumors that are locally controlled can be plotted as a function of dose, and data of this kind are amenable to a

sophisticated statistical analysis to determine the TCD<sub>50</sub>, the dose at which 50% of the tumors are locally controlled. This quantity is highly repeatable from one experiment to another in an inbred strain of animals. Suit and his colleagues, over more than 30 years, have made an extensive study of the response to radiation of a mammary carcinoma in C3H mice. Data from a typical experiment are presented in Figure 20.3. Tumors were propagated by transplanting  $4 \times 10^4$  cells into the

outer portion of the mouse ear, and irradiations were performed when the tumors had grown to a volume of about  $4 \text{ mm}^3$ . A brass circular clamp was fitted across the base of the ear and maintained for at least a minute before the initiation of the irradiation, so that the tumors were uniformly hypoxic. Single-dose, two-dose, and 10-dose experiments were performed, with a 24-hour interval between dose fractions. Tumor control results are shown in Figure 20.3. The TCD<sub>50</sub> for a

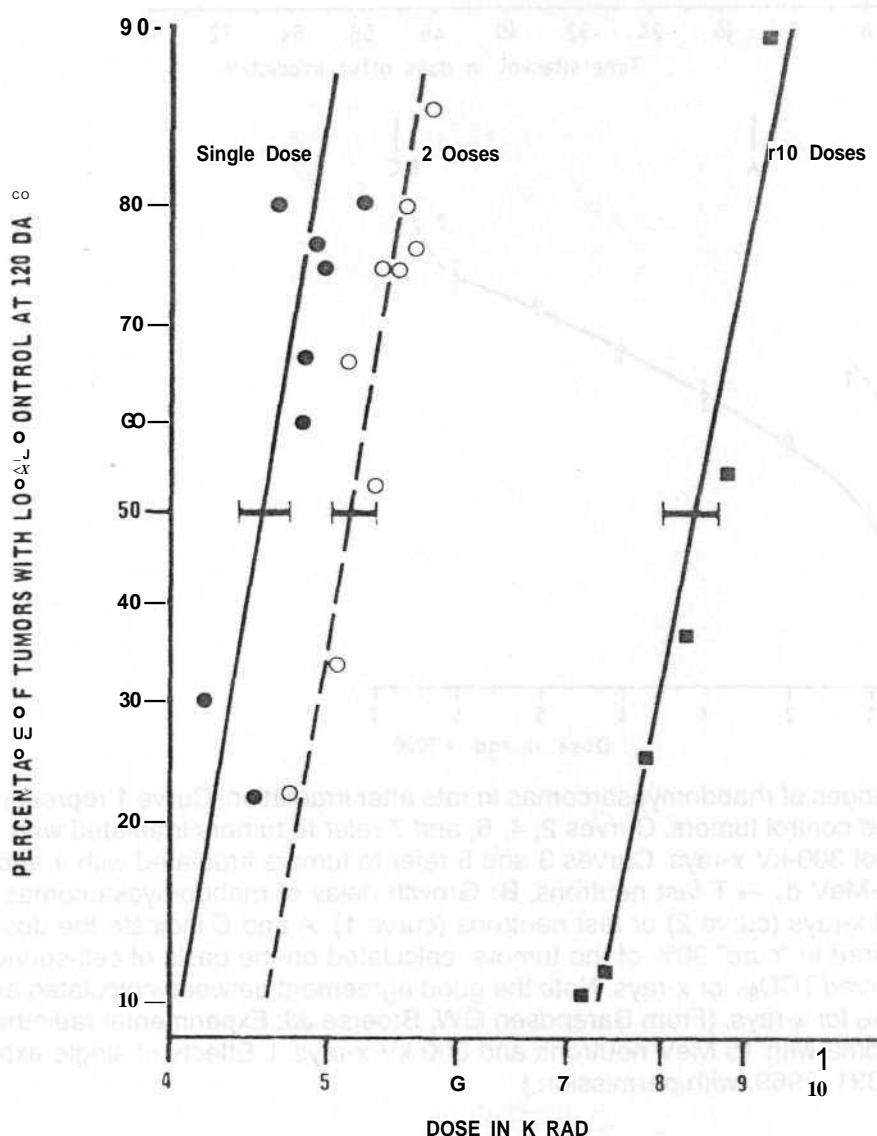


Figure 20.3. Percentage of mouse mammary tumors locally controlled as a function of x-ray dose, for single exposures and for two different fractionation patterns. The tumors were isotransplants derived from a spontaneous mammary carcinoma in a C3H mouse. The transplantation was made into the outer portion of the ear with  $4 \times 10^4$  viable cells. The tumors were treated when they reached a diameter of 2 mm (*i.e.*, a volume of about  $4 \text{ mm}^3$ ). (From Suit H, Wette R: Radiation dose fractionation and tumor control probability. *Radiat Res* 29:267-281, 1966, with permission.)

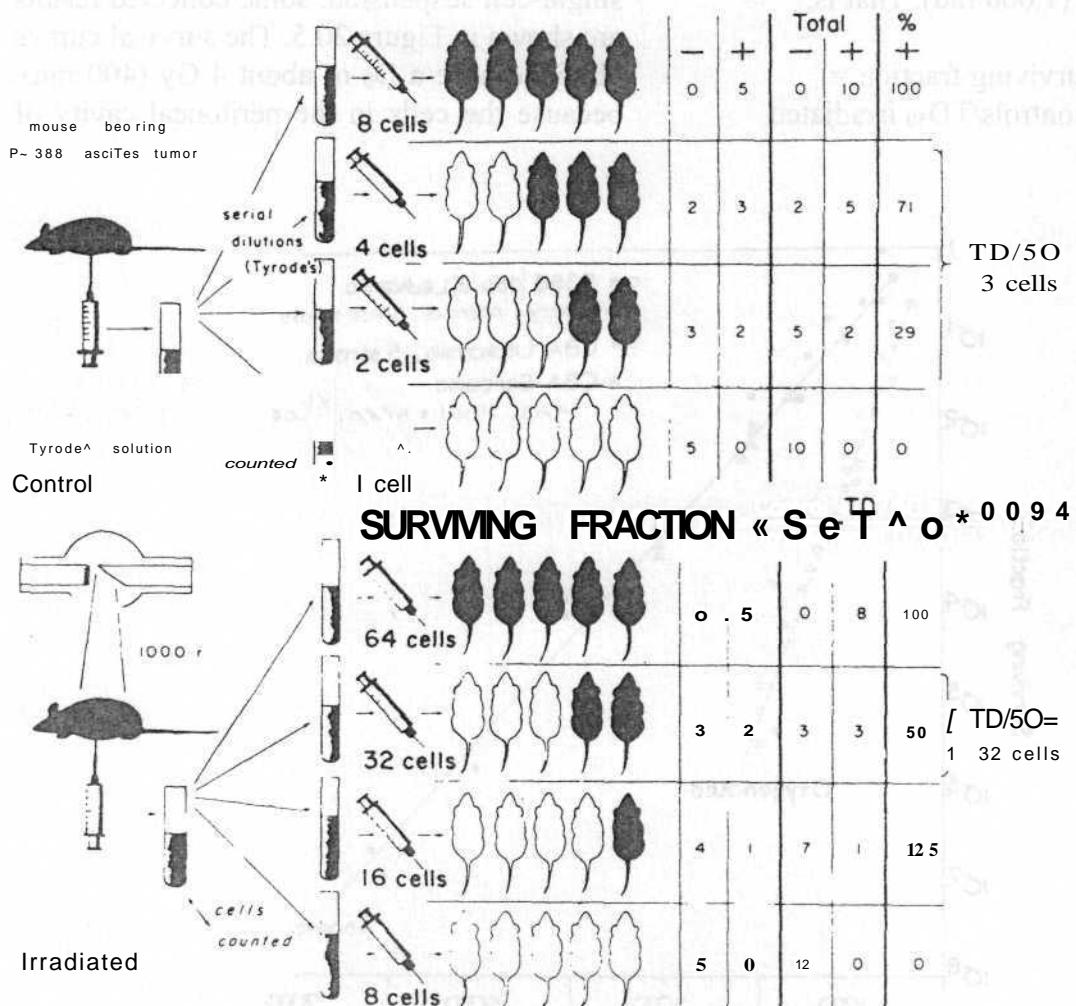
single treatment is 45.75 Gy (4,575 rad), rising to 51.1 Gy (5,110 rad) for two fractions and to 84 Gy (8,400 rad) if the radiation is delivered in 10 equal fractions. This indicates that a marked and extensive repair of sub-lethal damage has taken place during a multi-fraction regimen. Other examples of the use of this system are discussed in later chapters.

### DILUTION ASSAY TECHNIQUE

The **dilution** assay technique was devised by Hewitt and Wilson, who used it to produce the first *in vivo* survival curve in 1959. They used a lymphocytic leukemia of spontaneous

origin in mice. A single cell suspension can be prepared from the infiltrated liver of an animal with advanced disease and the tumor transplanted by injecting known numbers of cells into the peritoneal cavities of recipient mice, which subsequently develop leukemias. The leukemia can be transmitted, on average, by the injection of only two cells; this quantity—the number of cells required to transmit the tumor to 50% of the animals—is known as the TD50. The dilution assay technique became the basis for obtaining an *in vivo* cell survival curve.

The procedure used, illustrated in Figure 20.4, is as follows. An animal containing the



**Figure 20.4.** Schematic representation to show the general features of the dilution assay technique. Various numbers of tumor cells from the donor animal are injected into groups of recipients, and a determination is made of the number of cells required for a tumor to take in half of the animals of the group (TD50). The ratio of this quantity for control and irradiated donors is the surviving fraction. (From Andrew S Jr, Berry RJ: Radiat Res 16:76, 1962, with permission.)

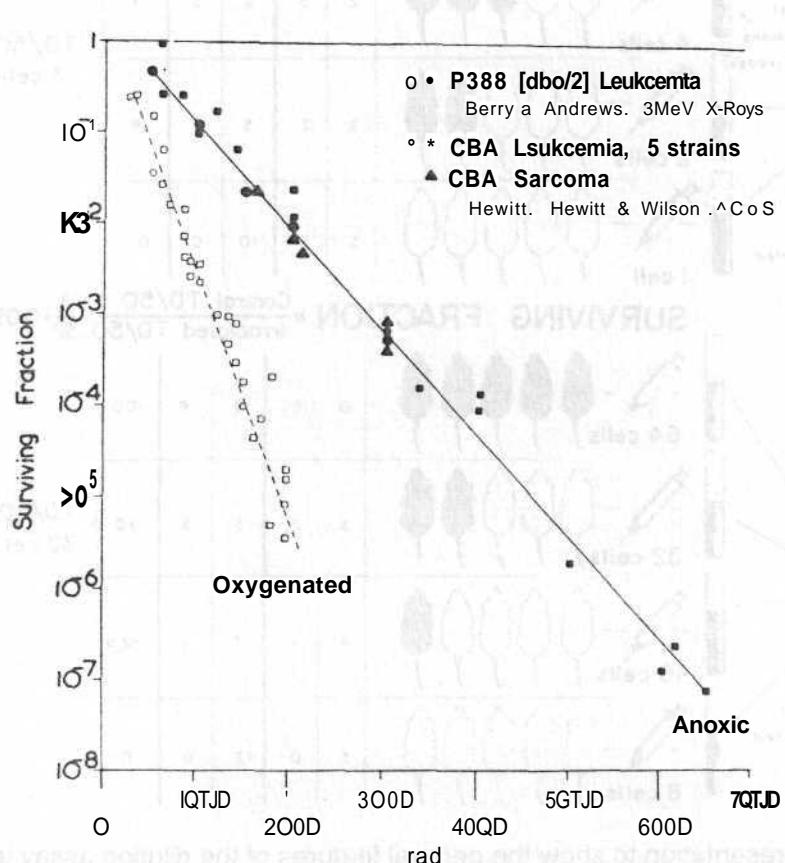
tumor may be irradiated to a given dose of radiation, for example, 10 Gy (1,000 rad). A single cell suspension then is prepared from the infiltrated liver, the cells are counted and diluted, and various numbers of these cells are injected intraperitoneally into groups of recipient animals. It is then a matter of observation and calculation to determine how many irradiated cells are required to produce a tumor in half of the animals inoculated with that given number of cells. Suppose, for instance, that it takes 20 irradiated cells, on the average, to transmit the tumor; because it is known that only two clonogenic cells are needed to transmit the tumor, it is a simple matter to decide that in the irradiated population of cells 2 of 20, or 10%, were clonogenic and survived the dose of 10 Gy (1,000 rad). That is,

$$\text{Surviving fraction} = \frac{\text{TD50 controls}}{\text{TD50 irradiated}}$$

If this process is repeated for a number of doses of radiation, and the corresponding surviving fractions are determined by this assay technique, a survival curve for cells irradiated *in vivo* can be constructed.

This technique is a true *in vivo* system, but it involves a leukemia as opposed to a solid tumor. The cells, after reinoculation into the mouse, grow in the peritoneal cavity in much the same way that the cells grow in a petri dish in the *in vitro* technique; the mice are in fact being used as small portable incubators.

Since these pioneering efforts, the dilution assay technique has been applied by many different workers to measure survival curves for a number of leukemias and solid tumors if the tumors can be removed and prepared into a single-cell suspension; some collected results are shown in Figure 20.5. The survival curves obtained have a Do of about 4 Gy (400 rad), because the cells in the peritoneal cavity of



**Figure 20.5.** Dose-response curves *in vivo*, using the dilution assay technique, for various murine tumors under oxygenated and hypoxic conditions. (From Berry RJ: Br J Radiol 37:948, 1964, with permission.)

the mouse are so numerous and so closely packed that they are deficient in oxygen. This technique, therefore, produces a "hypoxic" survival curve. To obtain a survival curve characteristic of aerated conditions, it is necessary either to remove the cells from the donor animal and irradiate them in a petri dish in which they are in contact with air, or to inject hydrogen peroxide into the peritoneal cavity of the mouse before irradiation, so that oxygen is available to the tumor cells during the irradiation. If this is done, the  $D_0$  is about 1.3 to 1.6 Gy (130-160 rad).

### LUNG COLONY ASSAY

Hill and Bush have devised a technique to assay the clonogenicity of the cells of a solid tumor irradiated *in situ* by injecting them into recipient animals and counting the number of lung colonies produced. The general principles of the method are illustrated in Figure 20.6. The tumor used in these studies was the KHT sarcoma, which is a transplantable tumor that arose originally in a C3H mouse, and which has been propagated serially through many generations. Tumors are irradiated *in situ*, after which they are removed and made into a preparation of single cells by a combined trypsinization and mechanical procedure. A known number of cells then is mixed with a large number of heavily irradiated tumor cells and injected intravenously into recipient mice. About 3 weeks later these mice

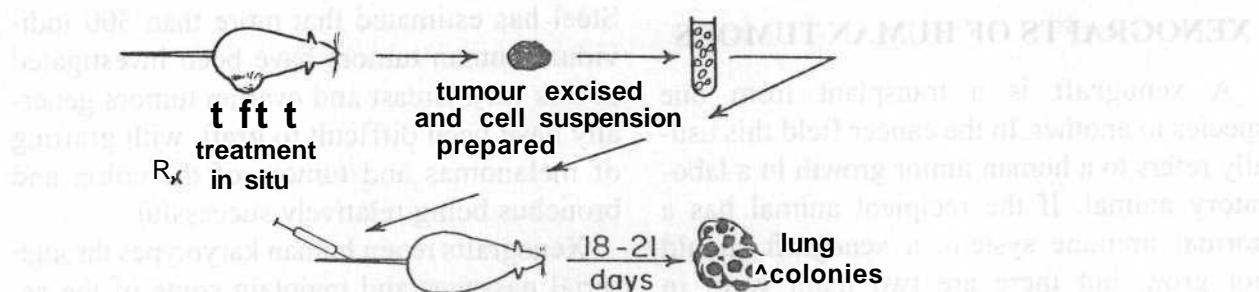
are sacrificed, and the colonies formed in the lungs are readily countable. The number of lung colonies is a measure of the number of surviving clonogenic cells in the injected suspension.

This technique was used for studies of dose rate described in Chapter 5. The lung colony technique is not confined to the KHT sarcoma but has been used with other tumor cells. For example, the demonstration of the absence of repair of potentially lethal damage after neutron irradiation involved the use of the Lewis lung carcinoma, and the fraction of surviving cells was assayed by counting lung colonies.

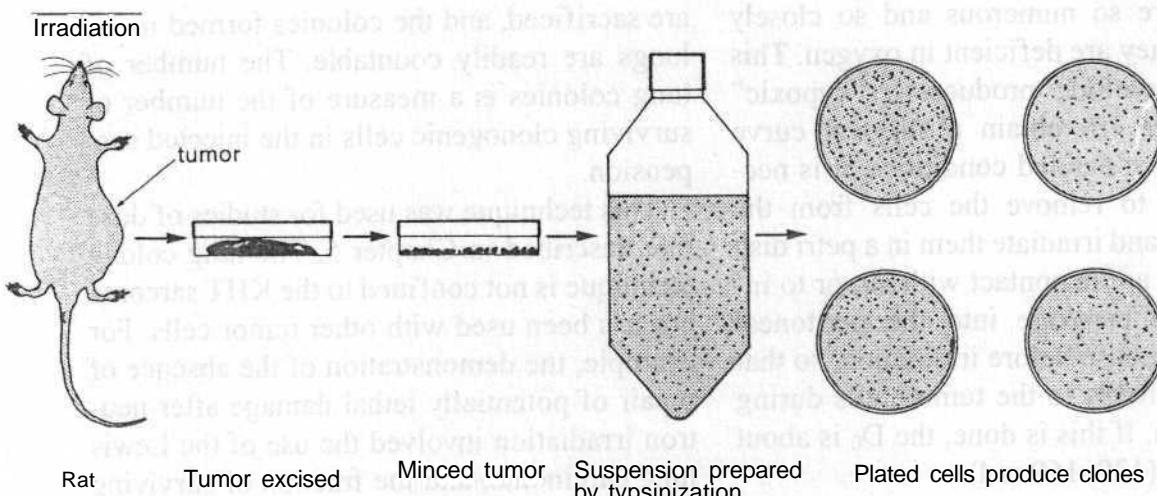
### IN. VIVO/IN VITRO TECHNIQUE

A limited number of cell lines have been adapted so that they grow either as a transplantable tumor in an animal or as clones in a petri dish. These cells can be readily transferred from *in vivo* to *in vitro* and back. In one generation they may grow as a solid tumor in an animal, and in the next as a monolayer in a petri dish. The three most commonly used systems are a rhabdomyosarcoma in the rat (Hermens and Barendsen), a fibrosarcoma in the mouse (McNally), and the EMT6 mammary tumor, also in the mouse (Rockwell and Kallman).

The steps involved in this method are illustrated in Figure 20.7. It combines many of the advantages of the *in vitro* and *in vivo* techniques. The tumors are treated *in vivo* in a nat-



**Figure 20.6.** The lung colony assay system. The tumor is irradiated *in situ*, after which it is excised and made into a single-cell suspension. A known number of cells then is injected intravenously into recipient animals. About 3 weeks later the recipient animals are sacrificed and the colonies that have formed in the lungs are counted. The number of lung colonies is a measure of the number of surviving clonogenic cells in the injected suspension. (From Hill RR Bush RS: Br J Radiol 46:167-174, 1973, with permission.)



**Figure 20.7.** The principle of the *in vivo/in vitro* assay system using the rhabdomyosarcoma in the rat. The solid tumor in the animal can be removed and the tumor cells assayed for colony formation in petri dishes. This cell line can be transferred to and fro between the animal and the petri dish. (Courtesy of Drs. G. W. Barendsen and J. J. Broerse.)

ural environment, so that the cellular response is modified by the various factors that are important in determining gross tumor response. After treatment, each tumor is removed and prepared into a single-cell suspension, and the cell concentration is counted in a hemacytometer or electronic cell counter. Known numbers of cells then can be transferred to petri dishes containing fresh growth medium, and the proportion of clonogenic cells can be determined by counting colonies 10 days later. The speed, accuracy, and relative economy of the *in vitro* system replaces the expense and inconvenience of the recipient animals in the dilution assay technique.

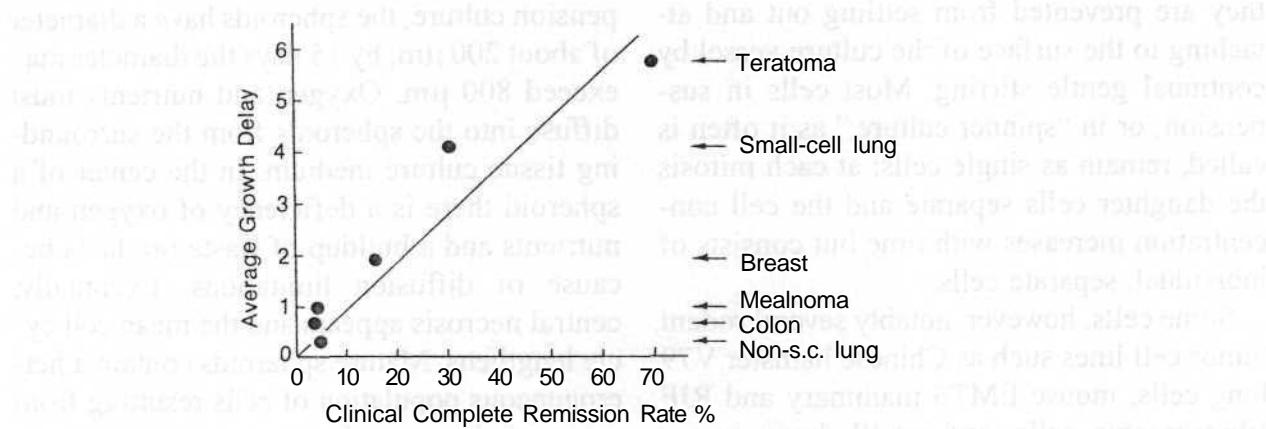
### XENOGRAFTS OF HUMAN TUMORS

A **xenograft** is a transplant from one species to another. In the cancer field this usually refers to a human tumor growth in a laboratory animal. If the recipient animal has a normal immune system, a xenograft should not grow, but there are **two** main ways in which growth has been achieved. First, animal strains have been developed that are congenitally immune-deficient. Best known are nude mice, which in addition to being hairless also lack a thymus. Many human tumors grow un-

der the skin of nude mice. More recently there have been nude rats and SCID mice, which suffer from the severe combined immunodeficiency syndrome and are deficient in both B-cell and T-cell immunity. Second, it is possible to severely immune-suppress mice by the use of radiation or drugs or a combination of both, to the point at which they accept human tumor grafts. It is important to recognize that neither type of host completely fails to reject the human tumor cells: Rejection processes are still present, and these complicate the interpretation of *in situ* tumor therapeutic studies.

Despite the limitations, a wide variety of human tumor cells have been grown as xenografts in immune-deficient animals. Steel has estimated that more than 300 individual human tumors have been investigated in this way. Breast and ovarian tumors generally have been difficult to graft, **with** grafting of melanomas, and tumors of the colon and bronchus being relatively successful.

Xenografts retain human karyotypes through serial passages and maintain some of the response characteristics of the individual source human tumors; to this extent, they have great advantages over mouse tumors. There are, however, certain drawbacks. First, there is a tendency for the tumor to be rejected, so that



**Figure 20.8.** Correlation between response of human tumor xenografts and clinical complete remission rates to chemotherapy. Ordinate is growth delay observed in 3 to 10 xenograft lines treated with the clinically used drugs that proved most effective in the xenografts. (Steel GG: How well do xenografts maintain the therapeutic response characteristics of the source tumor in the donor patient? In Kallman RF [ed]: Rodent Tumors in Experimental Cancer Therapy. New York, Pergamon, 1987, with permission.)

observing tumor control as an endpoint can be misleading. Growth-delay and cell-survival studies, on the other hand, are probably less affected. Second, human tumor cells do undergo kinetic changes and cell selection if transplanted into mice. For example, xenografts commonly have doubling times about one fifth of the values observed in humans, so that increased responsiveness should be expected to proliferation-dependent chemotherapeutic agents. Third, although the histologic characteristics of the human source tumors are usually well maintained by xenografts, the stromal tissue is of mouse origin. Consequently, xenografts of human tumor cells are not much more valid than murine tumors for any studies in which the vascular supply plays an important role. For example, the fraction of hypoxic cells in xenografts is much the same as in mouse tumors.

Steel and colleagues reviewed the field in 1983 and concluded that xenografts generally maintain the chemotherapeutic response characteristics of the class of tumors from which they are derived. There is good evidence, too, for individuality of response among xenografts. For example, in studying melanomas, one was responsive clinically, but another was not, and the cell-survival curve after therapy with melphalan was twice as steep in the xenograft of the cells from the responsive tumor.

Figure 20.8 summarizes the correlation between growth delay in the xenograft and clinical remission of the donor patient. In the figure, the growth delay in xenografts for maximum tolerated treatment with the single agents that are in common clinical use against the disease is plotted against clinical complete response rate for that category of tumor. The correlation between these parameters is good. Testicular tumors are the most responsive in xenografts or in the clinic; small-cell lung cancer and breast tumors occupy an intermediate position; and the other three tumor types are unresponsive, either clinically or experimentally. This consistency of agreement between patient and xenograft responses to chemotherapeutic agents is encouraging for a variety of human tumor types tested. Similarly, studies of radiation response indicate that measurements of growth delay in xenografts rank tumors in the same order as clinical responsiveness: Response is greater in testicular teratoma than in pancreatic carcinoma, which is greater than in bladder carcinoma, for example.

### SPHEROIDS: AN IN VITRO MODEL TUMOR SYSTEM

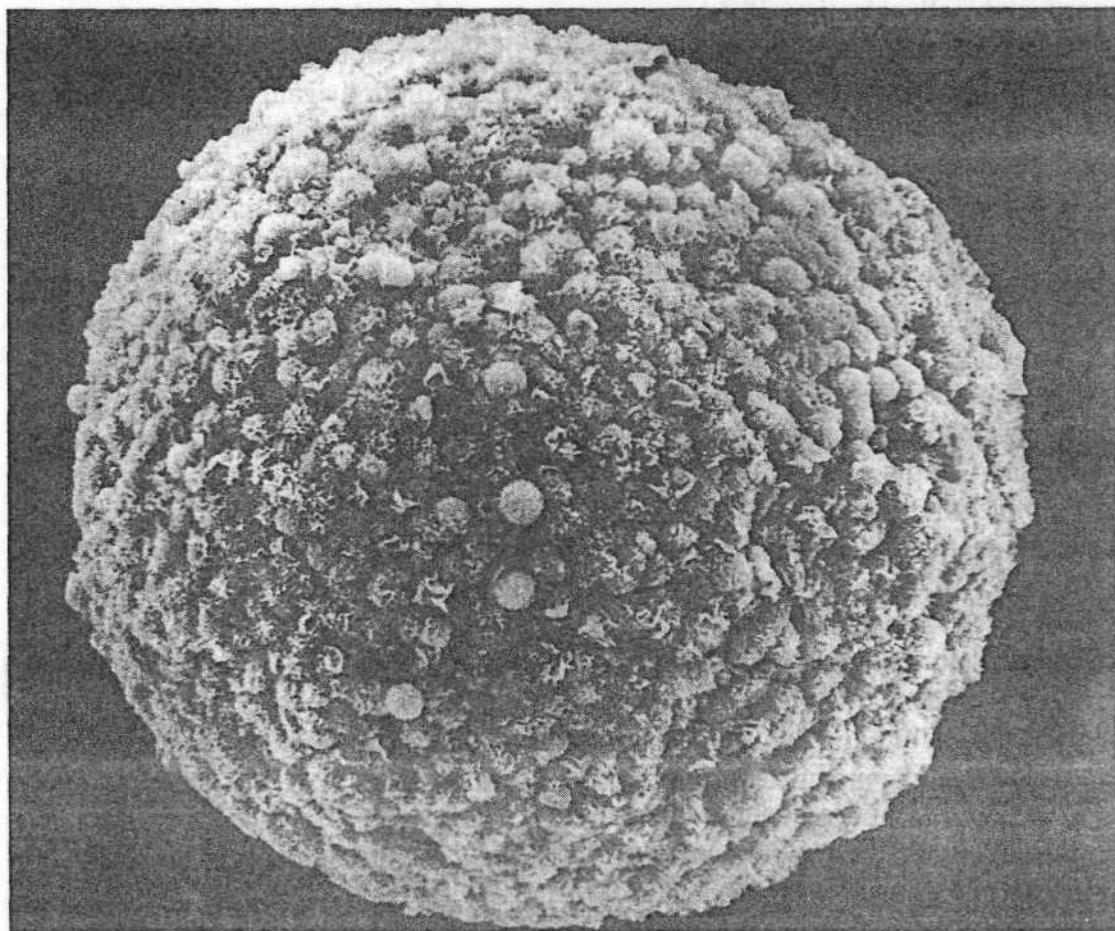
Mammalian cells in culture may be grown either as a monolayer attached to a glass or plastic surface or in suspension, in which case

they are prevented from settling out and attaching to the surface of the culture vessel by continual gentle stirring. Most cells in suspension, or in "spinner culture," as it often is called, remain as single cells; at each mitosis the daughter cells separate and the cell concentration increases with time but consists of individual, separate cells.

Some cells, however, notably several rodent tumor cell lines such as Chinese hamster V79 lung cells, mouse EMT6 mammary and RIF fibrosarcoma cells, and rat 9L brain tumor cells, do not behave in this way but instead grow as spheroids. At each successive division the daughter cells stick together, and the result is a large spheric clump of cells that grows bigger and bigger with time. A photograph of a large spheroid consisting of about  $8 \times 10^4$  cells is shown in Figure 20.9. Five days after the seeding of single cells into sus-

pension culture, the spheroids have a diameter of about 200  $\mu\text{m}$ ; by 15 days the diameter may exceed 800  $\mu\text{m}$ . Oxygen and nutrients must diffuse into the spheroids from the surrounding tissue culture medium. In the center of a spheroid there is a deficiency of oxygen and nutrients and a buildup of waste products because of diffusion limitations. Eventually, central necrosis appears and the mean cell cycle lengthens. Mature spheroids contain a heterogeneous population of cells resulting from many of the same factors, as in a tumor *in vivo*.

The spheroid system is simpler, more reproducible, less expensive, and easier to manipulate than animal tumors, and yet the cells can be studied in an environment that includes the complexities of cell-to-cell contact and nutritional stress from diffusion limitations that are characteristic of a growing tumor.



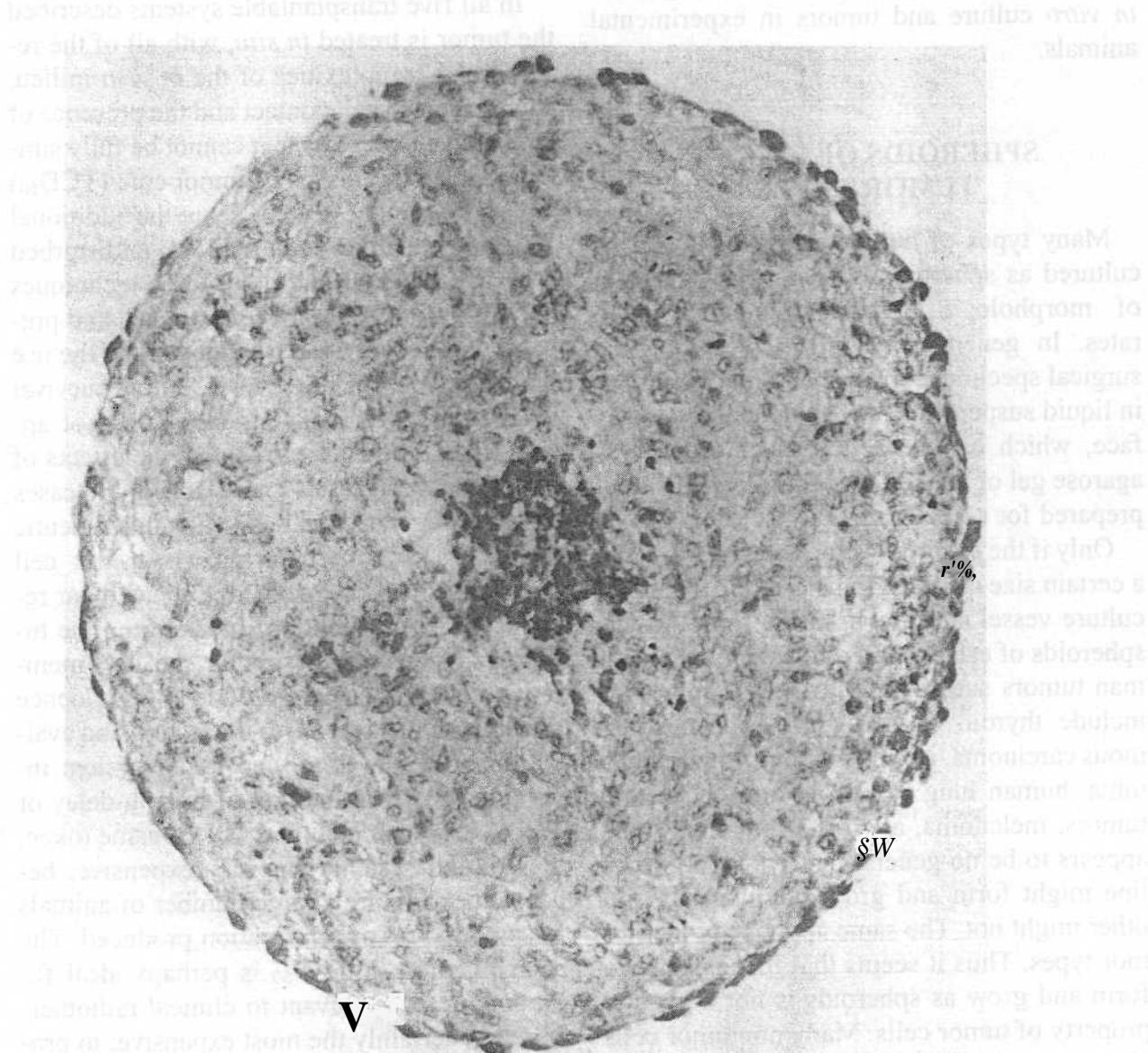
**Figure 20.9.** Photograph of an 800- $\mu\text{m}$  spheroid containing about  $8 \times 10^4$  cells. (Courtesy of Dr. R. M. Sutherland.)

Spheroids are irradiated intact and then separated into single cells by the use of trypsin and gentle agitation before being plated **out** into petri dishes to be assayed for the reproductive integrity of individual cells.

Mature spheroids consist of three populations of cells with varying radiosensitivity; starting from the outside and working toward the center, they are asynchronous, aerobic cycling cells; noncycling Gi-like cells; and non-cycling **Gi-like** hypoxic cells. Very large spheroids may contain about 20% hypoxic cells, similar to many animal tumors. By gently trypsinizing the spheroids for varying pe-

riods of time, the spheroid can be peeled like an onion and these three cell populations separated out. Using more sophisticated methods, such as centrifugal **elutriation** and flow cytometry, it is possible to separate many more cell subpopulations based on location in the spheroid, cell cycle, or other parameters. Figure 20.10 is a cross-section through a large spheroid, showing clearly the development of a central necrotic area if its size is such that oxygen and other nutrients cannot diffuse into **the center**.

The spheroid system has been applied to a number of problems in radiobiology and in



**Figure 20.10.** Photomicrograph of a spheroid. Note the area of central necrosis. The spheroid was grown for 15 days and was 520  $\mu\text{m}$  in diameter; the viable rim had an average thickness of about 200  $\mu\text{m}$ . (Courtesy of Dr R. M. Sutherland.)

the study of pharmacologic agents, such as radiosensitizers or chemotherapeutic agents. A major problem in the application of these drugs to human tumors is the presence of resistant cells that are resting or noncycling, often located away from blood vessels. Drugs are required to diffuse in effective concentration to these cells through layers of growing actively dividing cells, which may inactivate the drug through their metabolism. The spheroid system mimics many of these tumor characteristics and provides a rapid, useful, and economic method for screening sensitizers and chemotherapeutic agents because it is intermediate in complexity between single-cell *in vitro* culture and tumors in experimental animals.

### SPHEROIDS OF HUMAN TUMOR CELLS

Many types of human tumor cells can be cultured as spheroids, with a wide spectrum of morphologic appearances and growth rates. In general, cells from disaggregated surgical specimens form spheroids if cultured in liquid suspension above a nonadhesive surface, which can be a thin layer of agar or agarose gel or the bottom of a culture dish not prepared for cell culture.

Only if the spheroid is formed and grown to a certain size can it be transferred to a spinner culture vessel and grown in the same way as spheroids of established rodent cell lines. Human tumors successfully grown as spheroids include thyroid cancer, renal cancer, squamous carcinoma, colon carcinoma, neuroblastoma, human lung cancer, glioma, lymphoid tumors, melanoma, and osteosarcoma. There appears to be no general pattern. One glioma line might form and grow as spheroids; another might not. The same applies to other tumor types. Thus it seems that the capacity to form and grow as spheroids is not a general property of tumor cells. Many nontumor cells also form spheroids, but only the spheroids of lymphoid origin continue to grow to any size.

Morphologic studies of spheroids of human tumor cells show that they maintain many

characteristics of the original tumor specimens taken from the patient and of the cells if grown as a xenograft in nude mice. Radiobiologic studies show that, in addition to maintaining histologic characteristics of individual tumors, spheroids of human cells preserve characteristic radiosensitivity, because dose-response curves for spheroids are virtually identical to those for cells growing as xenografts in nude mice.

### COMPARISON OF THE VARIOUS MODEL TUMOR SYSTEMS

In all five transplantable systems described the tumor is treated *in situ*, with all of the realism and complexities of the *in vivo* milieu, such as cell-to cell contact and the presence of hypoxic cells, factors that cannot be fully simulated in a petri dish. The tumor cure (**TCD<sub>50</sub>**) and growth delay systems share the additional advantage that they are left *in situ* undisturbed after treatment. In the other three techniques the tumor must be removed, minced, and prepared into a single-cell suspension by the use of an enzyme, such as trypsin, before survival is assessed. Although this step does not appear to affect the assessment of the effects of radiation, it can result in artifacts in the cases of other agents, such as chemotherapeutic drugs or hyperthermia, in which the cell membrane may be involved in the cellular response. The procedure of breaking up the tumor and partially dissolving the cell membrane with a digestive enzyme may influence results. For this reason, in the testing and evaluation of a new drug, one tumor system involving the determination of growth delay or **TCD<sub>50</sub>** is always included. By the same token, these same systems are very expensive, because they require a large number of animals for the amount of information produced. The determination of **TCD<sub>50</sub>** is perhaps ideal for producing data relevant to clinical radiotherapy. It is certainly the most expensive; to produce a single **TCD<sub>50</sub>** value for one of the lines in Figure 20.3, six to eight groups of up to 10 animals must be kept and observed for weeks. The same information can be obtained in 10

days with one or two mice and six petri dishes using the *in vivo/in vitro* technique.

The dilution assay technique allows clonogenic cell survival to be assessed over a large range of doses and for tumors that cannot be grown in culture. It, too, is relatively expensive, because a whole group of recipient animals must be used and kept for weeks to obtain the same information obtained from one petri dish. Unquestionably, the most rapid and efficient technique is the *in vivo/in vitro* technique, which combines the realism of irradiation *in vivo* with the speed and efficiency of *in vitro* plating to assess clonogenic survival. The concomitant disadvantage is that any tumor that can be switched from petri dish to animal in alternate passages is so undifferentiated and anaplastic that it bears little resemblance to a spontaneous tumor in the human.

To some extent, the same criticism can be levied at all transplantable tumor systems. They are highly quantitative, but they are also very artificial. Having been passaged for

many generations, they tend to be highly undifferentiated, and they grow as encapsulated tumors in a muscle or beneath the skin rather than in the tissue of origin. In addition, some have produced misleading results because they are highly antigenic, which, in general, human tumors are not.

In short, transplantable tumors in laboratory animals are model systems; they must be used with care, and the results must not be overinterpreted. Used with caution, these systems have provided invaluable quantitative data and helped to establish important radiobiologic principles. They also, however, have "led us up the garden path" on several occasions in the past (Fig. 20.11). For all of the reasons listed previously, they differ in important ways from spontaneous human tumors, and for the testing of drugs at the National Cancer Institute they have been largely replaced by a battery of cells of human origin cultured *in vitro*.

Xenografts of human tumors so far have been used on a much more limited scale.

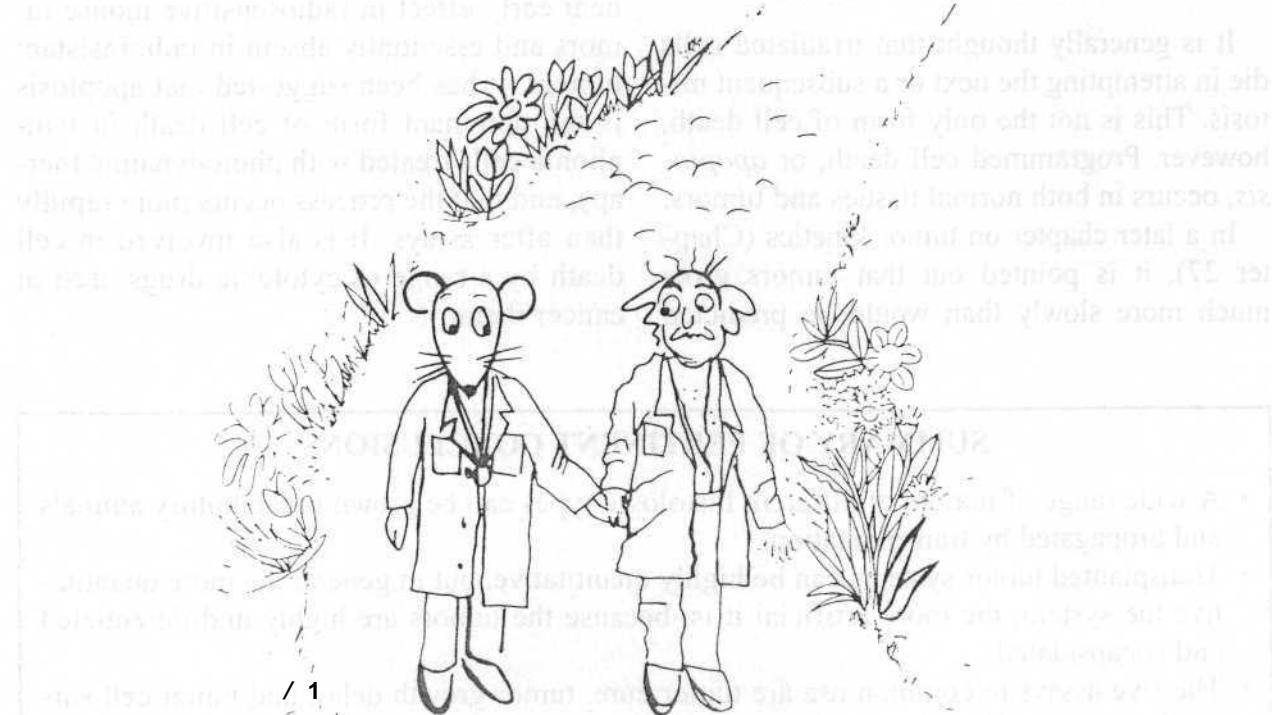


Figure 20.11. Transplantable tumors in small laboratory animals have provided invaluable quantitative data, but they also have "led us up the garden path" on several occasions. Transplantable tumors tend to be fast growing, undifferentiated, and highly antigenic and to grow as encapsulated tumors in muscle or beneath the skin, not in their sites of origin. For all of these reasons they are highly artificial, and care must be used in interpreting results.

Because they are grown in the absence of an immune response, it could be argued that they are the epitome of artificiality. They do, however, allow a comparison to be made of the intrinsic sensitivity to radiation or chemotherapeutic agents of fresh explants of human tumors. As *in vitro* culture techniques improve and better growth media are developed, xenografts may be less necessary.

Spheroids represent a most important intermediate model between monolayers of cells in culture and tumors *in vivo*. A number of important radiobiologic principles have been established using spheroids of rodent cells, in which the various populations of cells, aerated *versus* hypoxic or cycling *versus* noncycling, can be separated out. Human cell spheroids have only been used on a limited scale, but it is clear that the cells retain many of the characteristics of the tumor from which they were taken. Spheroids are much less expensive than xenografts in immunosuppressed animals and perform much the same function.

### APOPTOSIS IN TUMORS

It is generally thought that irradiated cells die in attempting the next or a subsequent mitosis. This is not the only form of cell death, however. Programmed cell death, or *apoptosis*, occurs in both normal tissues and tumors.

In a later chapter on tumor kinetics (Chapter 27), it is pointed out that tumors grow much more slowly than would be predicted

from the cell cycle time of the individual cells and the fraction of cells actively dividing. One of the reasons for this "cell loss," as it is called, is random cell death resulting from apoptosis.

Studies with transplanted mouse tumors, as well as human tumors growing as xenografts in nude mice, have shown that the importance of apoptosis as a mechanism of cell death after x-irradiation varies substantially. Apoptosis was most important in lymphomas, essentially absent in sarcomas, and intermediate and very variable for carcinomas. In a mouse lymphoma, for example, 50 to 60% of the cells may show signs of dying an apoptotic death by 3 hours after irradiation, whereas in a sarcoma there may be so few apoptotic cells that the process is of little significance. If a tumor responds rapidly to a relatively low dose of radiation, it generally means that apoptosis is involved, because the process peaks at 3 to 5 hours after irradiation. Susceptibility to the induction of apoptosis also may be an important factor determining radiosensitivity, because programmed cell death appears to be a prominent early effect in radiosensitive mouse tumors and essentially absent in radioresistant tumors. It has been suggested that apoptosis is the dominant form of cell death in lymphoma cells treated with photodynamic therapy, and that the process occurs more rapidly than after x-rays. It is also involved in cell death by a range of cytotoxic drugs used in cancer therapy.

### SUMMARY OF PERTINENT CONCLUSIONS

- A wide range of tumors of different histologic types can be grown in laboratory animals and propagated by transplantation.
- Transplanted tumor systems can be highly quantitative, but in general the more quantitative the system, the more artificial it is, because the tumors are highly undifferentiated and encapsulated.
- The five assays in common use are tumor cure, tumor growth delay, and tumor cell **survival** determined by the dilution assay technique, the production of lung colonies, or colonies *in vitro*.
- In all five assays the cells can be irradiated *in situ* with all the realism and complexity of *in vivo* conditions.

- If tumor "cure" (TCD50) or growth delay is scored, the tumor is left undisturbed after treatment. This avoids artifacts involved in disaggregating the tumor, especially in the study of some chemicals or hyperthermia, in which cell membrane effects are important.
- The dilution assay technique, the lung colony assay, and the *in vivo/in vitro* assay all measure cell surviving fraction; that is, they are clonogenic assays. They require fewer animals and are therefore more efficient than the scoring of tumor cure or growth delay. All three assays require, however, that a single cell suspension be prepared from the tumor, and this may result in artifacts.
- Transplantable tumors in small laboratory animals have been used to establish many radiobiologic principles; but they are highly artificial and must be used with care. They have "led us up the garden path" on several occasions.
- Many human tumor cells can be grown as xenografts in immune-deficient animals.
- Although the histologic characteristics of the human source tumor are maintained, the stroma is of mouse origin.
- Xenografts of human tumor cells are not much better than mouse tumors for studies in which the vascular supply is important.
- Human tumor cells undergo kinetic changes and selection if transplanted into immune-deficient mice.
- Xenografts generally maintain the chemotherapeutic response characteristics of the class of tumors from which they are derived. There is evidence, too, of individuality of response.
- Spheroids of established rodent cells can be grown in suspension culture (*i.e.*, "spinner culture"). Oxygen and nutrients must diffuse into the spheroid from the surrounding culture medium. Oxygen deficiency and a buildup of waste products result, just as in a tumor.
- Mature spheroids contain a heterogeneous population of cells, much like a tumor, but are more quantitative and more economical to work with.
- Starting from the outside and working toward the center, spheroids consist of asynchronous aerated cells, noncycling G<sub>1</sub>-like aerated cells, noncycling G<sub>2</sub>-like hypoxic cells, and necrotic cells.
- Spheroids are intermediate in complexity between monolayer cell cultures *in vitro* and transplantable tumors in animals.
- Many types of human tumor cells grow as spheroids and maintain many characteristics of the original tumor from the patient or of the same cells grown as xenografts.
- Programmed cell death, or apoptosis, occurs in many animal tumors after irradiation, as well as mitotic death.
- Cells may show signs of dying an apoptotic death by 3 hours after irradiation.
- Apoptosis is most important in lymphomas, essentially absent in sarcomas, and intermediate and variable in carcinomas.

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## Cell, Tissue, and Tumor Kinetics

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### THE CELL CYCLE

#### QUANTITATIVE ASSESSMENT OF THE CONSTITUENT PARTS OF THE CELL CYCLE

#### THE PERCENT-LABELED MITOSES TECHNIQUE

#### EXPERIMENTAL MEASUREMENTS OF CELL CYCLE TIMES *IN VIVO* AND *IN VITRO*

#### PULSED PHOTO CYTOMETRY

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#### THE OVERALL PATTERN OF TUMOR GROWTH

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#### COMPARISON OF THE CELL CYCLE TIMES OF THE CELLS OF SOLID TUMORS AND THEIR NORMAL COUNTERPARTS

#### SUMMARY OF PERTINENT CONCLUSIONS

### THE CELL CYCLE

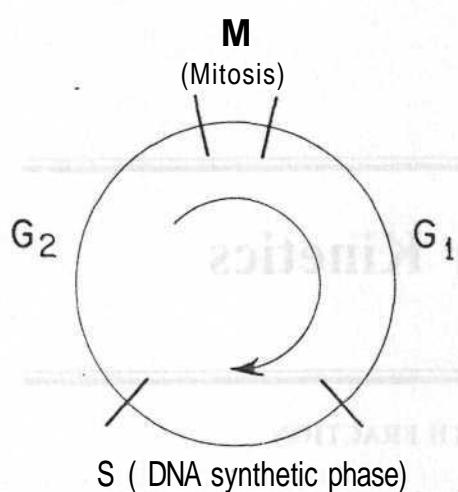
Mammalian cells replicate and increase in number by mitosis. If growing cells are observed with a conventional light microscope, the only event that can be distinguished is the process of mitosis itself. For most of the cell cycle the chromosomes are diffuse and not clearly seen, but for a short time before mitosis they condense into discrete and recognizable entities. There is a brief flurry of activity as the chromosomes separate into two groups and move to the two poles of the cell; division then occurs to form two daughter cells. The average interval between successive mitoses or divisions is called the **cell cycle** or **mitotic cycle time** ( $T_c$ ).

Howard and Pelc were the first to further subdivide the mitotic cycle by the use of a labeled DNA precursor. This is described in Chapter 4 and is reviewed only briefly here. It is a simple matter to identify the proportion of cells synthesizing DNA (*i.e.*, in S phase). The DNA precursor is made available to a grow-

ing population of cells, **in** which it is taken up and incorporated into the DNA of cells that are actively synthesizing DNA at that time. Cells that are not making DNA do not take up the label. The cell preparation then is fixed and stained and viewed through a microscope. The DNA precursor may be thymidine labeled with radioactive tritium, which may be identified later by autoradiography, or 5-bromo-deoxyuridine, which may be identified later by the use of a specific **stain** or antibody.

These labeling techniques can be applied to cells growing *in vitro* in petri dishes or to tissues *in vivo*, if, after the incorporation of the label, the tissue or tumor of interest is removed and sliced into sections a few microns thick.

By the use of these techniques, the cell cycle in all dividing mammalian cells may be divided as shown in Figure 21.1. After the cells pass through mitosis (M), there is a period of apparent inactivity, termed Gi by Howard and Pelc, simply because it was the first "gap" in activity observed in the cell cycle. After this



**Figure 21.1.** The phases of the cell cycle. Mitosis (M) is the only event that can be distinguished through the light microscope. The DNA synthetic phase (S) may be identified by the technique of autoradiography (Chapter 4). The intervals of apparent inactivity are labeled G<sub>1</sub> and G<sub>2</sub>.

period of inactivity, the cells actively synthesize DNA during S phase. Between S phase and the onset of the next division or mitosis, there is another gap in activity, termed G<sub>2</sub>.

#### QUANTITATIVE ASSESSMENT OF THE CONSTITUENT PARTS OF THE CELL CYCLE

Two **relatively** simple measurements can be made on a population of cells. First, it is possible to count the proportion of cells that are seen to be in mitosis; this quantity is called the **mitotic index** (MI). If it is assumed that all of the cells in the population are dividing, that all of the cells have the same mitotic cycle, then,

$$MI = X \frac{TM}{TC}$$

in which TM is the length of mitosis (*i.e.*, the time taken for cells to complete division), and TC is the total length of the mitotic or cell cycle.

The X is a correction to allow for the fact that cells cannot be distributed uniformly in time around the cycle, because they double during mitosis (Fig. 21.2). The simplest as-

sumption is that cells are distributed around the cycle exponentially in time, **in** which case X has a value of 0.69. At all events, the X is a relatively small and unimportant correction factor.

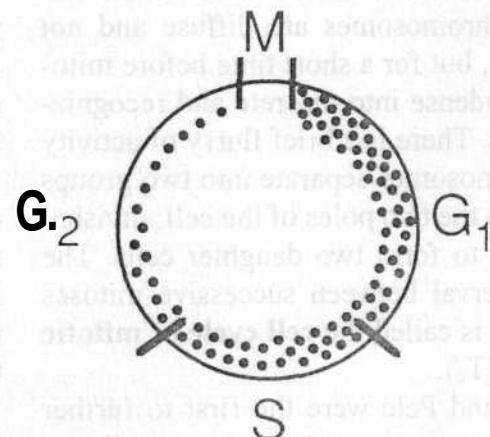
The second relatively simple measurement requires that the cell population be fed for a brief time with a quantity of tritiated thymidine or bromodeoxyuridine. In the jargon of cell kinetics, it is said to be **flash-labeled**. The cell population, whether on a **petri** dish or in a thin section cut from tissue, then is fixed, stained, and viewed through a microscope. A count is made of the proportion of labeled cells. This quantity is called the **labeling index** (LI).

Given the assumption that all the cells are dividing with the same cell cycle, then

$$LI = X Ts/Tc$$

in which Ts is the duration of the DNA synthetic period, and Tc is the total cell cycle time.

In practice these two quantities—the mitotic index and the labeling index—can be determined from a single specimen by counting the proportion of cells in mitosis and the proportion of cells that are labeled. This is a very important consideration in human stud-



**Figure 21.2.** Diagram illustrating the fact that cells cannot be distributed uniformly in time around the cell cycle because they double in number during mitosis. The simplest assumption is that they are distributed as an exponential function of time.

ies, in which it is usually not practical to obtain a large number of serial specimens of tumor or normal tissue material. Although these measurements yield ratios of the duration of mitosis and DNA synthesis as fractions of the total cell cycle, they do not give the absolute duration in hours of any part of the cycle.

### THE PERCENT-LABELED MITOSES TECHNIQUE

A complete analysis of the cell cycle to obtain the length of each phase is only possible by labeling a cohort of cells in one phase of the cycle and observing the progress of this labeled cohort through a "window" in some other readily observable phase of the cycle. In practice the easiest phase to label is S, and the easiest to observe is M.

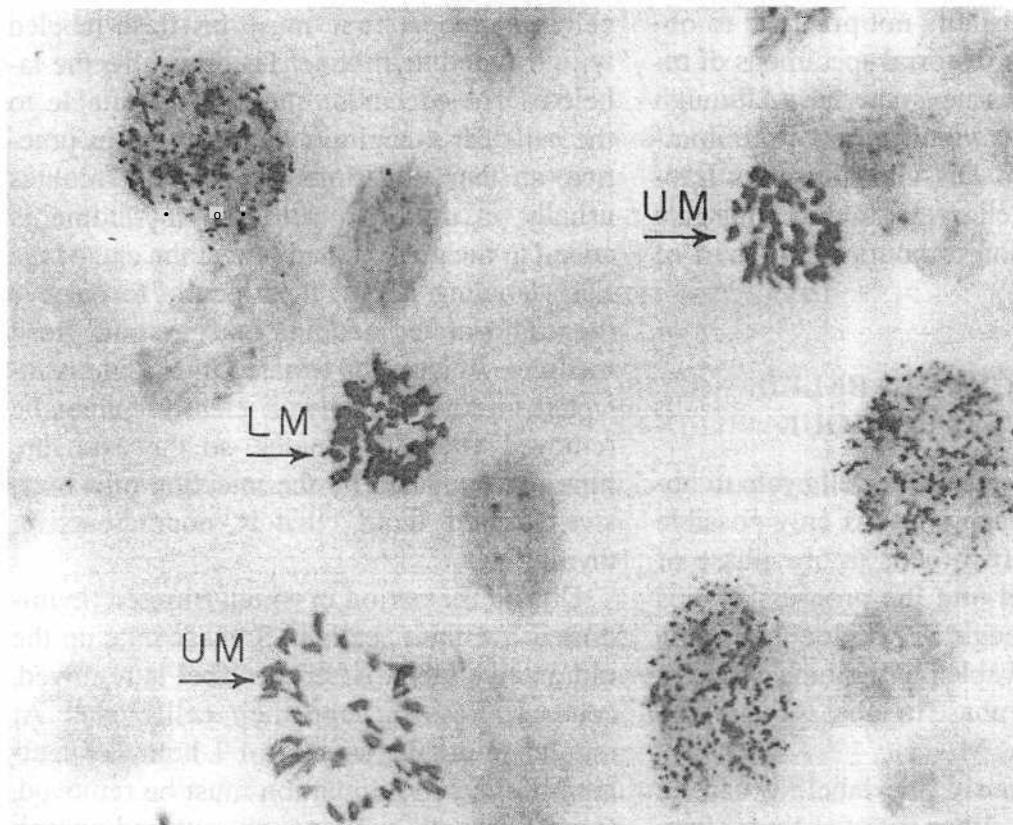
As stated previously, the labeling can be achieved by using either tritiated thymidine, identifiable by autoradiography, or bromodeoxyuridine, identifiable by a specific stain or antibody. The basis of the technique, therefore, is to feed the population of cells a label that is taken up in S, and then to observe the appearance of that label in mitotic cells as they move around the cycle from S to M. To avoid confusion, the technique involving tritiated thymidine is described in detail, partly because it is the original and classic technique and partly because pictures of autoradiographs show up well in black and white. The technique works equally well if bromodeoxyuridine is used. Bromodeoxyuridine-containing DNA can be stained, and shows up well in color under a microscope, but does not reproduce well in black and white.

The percent-labeled mitoses technique is laborious and time-consuming and requires a large number of serial samples. It is readily applicable *in vitro*, for which it is not difficult to obtain a large number of parallel replicate samples. It also may be applied *in vivo* for determining the cell-cycle parameters of normal tissue or tumors, provided a large number of sections from matched animals or tumors can be obtained at accurately timed intervals. The

cell population first must be flash-labeled with tritiated thymidine. Theoretically, the labeled DNA precursor should be available to the cells for a negligibly short time; in practice, an exposure time of about 20 minutes usually is used. *In vitro* the thymidine is added to the growth medium; at the end of the flash-labeling period it is simple to remove the radioactive medium and to add fresh medium. *In vivo*, the tritiated thymidine is injected intraperitoneally; it clearly cannot be removed after 20 minutes, so the exposure time is terminated by the injection of a massive dose of "cold," that is, nonradioactive, thymidine.

During the period in which tritiated thymidine is available, cells in S phase take up the radioactive label. After the label is removed, cells progress through their cell cycles. At regular intervals, usually of 1 hour, a specimen of the cell population must be removed, fixed, and stained and an autoradiograph prepared. This is continued for a total time longer than the cell cycle of the population under study. For each sample the percentage of mitotic cells that carry a radioactive label must then be counted; this is the **percentage of labeled mitoses**. A photomicrograph of a cell preparation is shown in Figure 21.3. This is a particularly laborious process, because only 1 or 2% of the cells are in mitosis in any case, and only a fraction of these will be labeled.

The basis for this type of experiment, if applied to an idealized population of cells that all have identical cell cycles, is illustrated in Figure 21.4, a plot of the percentage of labeled mitoses as a function of time. The cells that are in S while the radioactive thymidine is available take up the label. This labeled cohort of cells then moves through the cell cycle (as indicated by the circles at the top of Fig. 21.4) after the pool of radioactive thymidine has been removed. Samples obtained in the first few hours contain no labeled mitotic figures, and the first labeled mitotic figure appears as the leading edge of the cohort of labeled cells reaches M. This point in time is labeled b on the time axis of Figure 21.4; the position of



**Figure 21.3.** Photomicrograph of a preparation of mouse corneal cells. The cell preparation was flash-labeled some hours before with tritiated thymidine, which was taken up by cells in S. By the time the autoradiograph was made, the cell marked LM had moved around the cycle into mitosis; this is an example of a labeled mitotic figure. Other cells in mitosis are not labeled (UM). (Courtesy of Dr. M. Fry.)

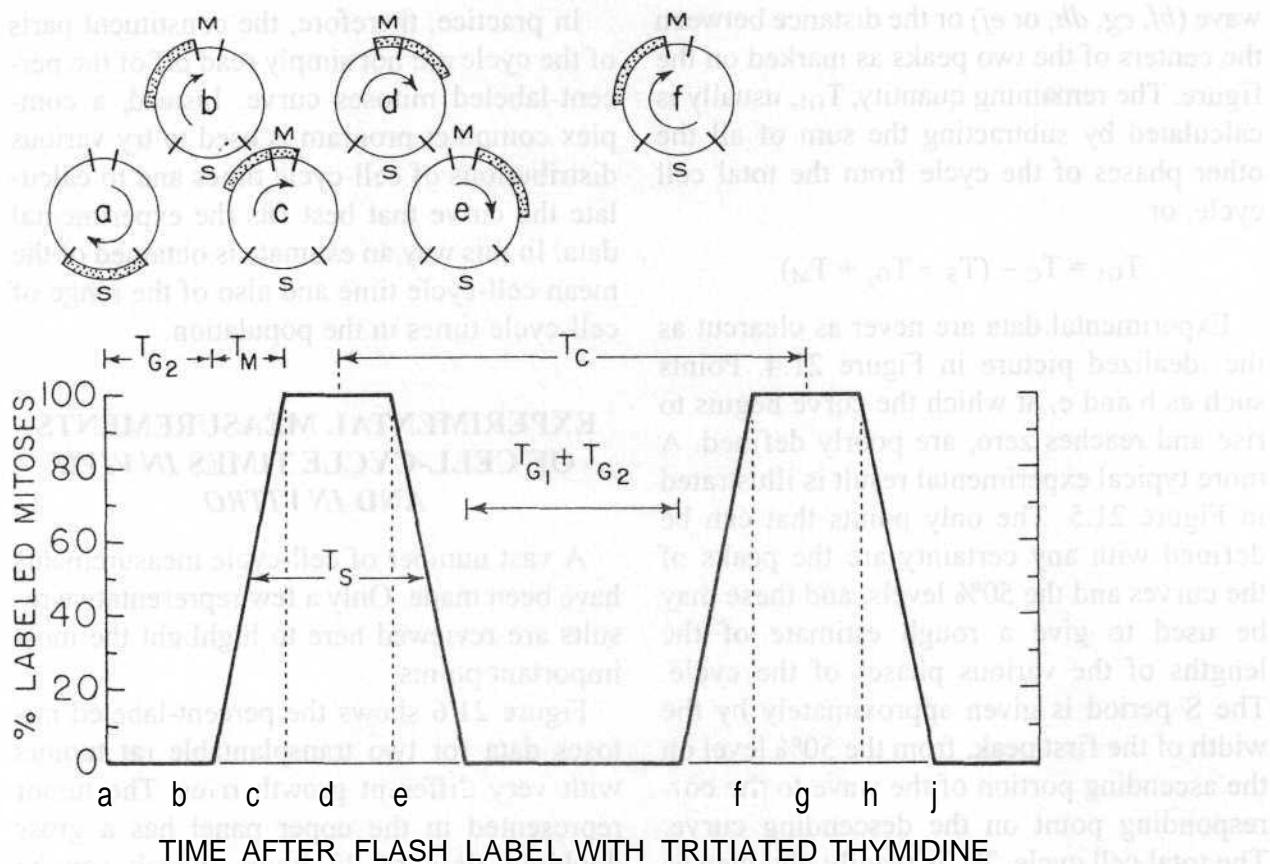
the labeled cohort is indicated above on the circle also marked b.

The percentage of mitotic figures labeled increases rapidly as the leading edge of the labeled cohort of cells passes through the M phase; when it reaches the end of the M phase, all mitotic figures are labeled (see position c). For the next few hours all mitotic figures continue to be labeled until the trailing edge of the labeled cohort of cells reaches the beginning of mitosis (see position d), after which the percentage of labeled mitoses rapidly falls and reaches zero when the trailing edge reaches the end of mitosis (see position e).

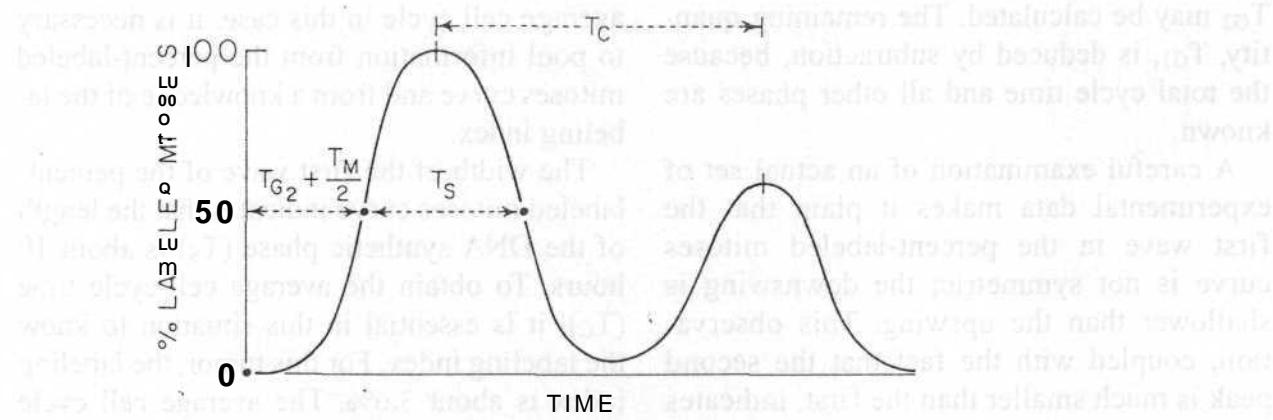
There then is a long interval during which no labeled mitotic figures are seen until the labeled cohort of cells goes around the entire cycle and comes up to mitosis again, after which the whole pattern of events is repeated.

All of the parameters of the cell cycle may be calculated from Figure 21.4. The time interval before the appearance of the first labeled mitosis, the length  $ah$ , is in fact the length of G2 or TG2- The time it takes for the percent-labeled-mitoses curve to rise from 0% to 100% (*be*) corresponds to the time necessary for the leading edge of the labeled cohort of cells to proceed through mitosis and is therefore equal to the length of mitosis,  $TM$ . The duration of DNA synthesis ( $Ts$ ) is the time taken for the cohort of labeled cells to pass the beginning of mitosis (*bd*). Likewise, it is the time required for the labeled cohort to pass the end of mitosis, *ce*. In practice,  $Ts$  usually is taken to be the width of the curve at the 50% level, as marked in Figure 21.5.

The total cycle ( $Tc$ ) is the distance between corresponding points on the first and second



**Figure 21.4.** Percent-labeled mitoses curve for an idealized cell population in which all of the cells have identical mitotic cycle times. The cell population is flash-labeled with tritiated thymidine, which labels all cells in S. The proportion of labeled mitotic cells is counted as a function of time after labeling. The circles at the top of the figure indicate the position of the labeled cohort of cells as it progresses through the cycle. The length of the various phases (e.g.,  $T_{G_2}$ ,  $T_M$ ) of the cycle ( $T_C$ ) may be determined as indicated.



**Figure 21.5.** Typical percent-labeled mitoses curve obtained in practice for the cells of a tissue or tumor. It differs from the idealized curve in Figure 21.4 in that the only points that can be identified with precision are the peaks of the curve and the 50% levels. The first peak is symmetric, and the second peak is lower than the first because the cells of a population have a range of cell cycle times.

wave (*bf*, *eg*, *dh*, or *ej*) or the distance between the centers of the two peaks as marked on the figure. The remaining quantity, TGI, usually is calculated by subtracting the sum of all the other phases of the cycle from the total cell cycle, or

$$T_{GI} = T_C - (T_S + T_{G_2} + T_M)$$

Experimental data are never as clearcut as the idealized picture in Figure 21.4. Points such as *b* and *e*, at which the curve begins to rise and reaches zero, are poorly defined. A more typical experimental result is illustrated in Figure 21.5. The only points that can be defined **with** any certainty are the peaks of the curves and the 50% levels, and these may be used to give a rough estimate of the lengths of the various phases of the cycle. The S period is given approximately by the width of the first peak, from the 50% level on the ascending portion of the wave to the corresponding point on the descending curve. The total cell cycle, *T<sub>c</sub>*, is readily obtained as the time between successive peaks. In a separate experiment the mitotic index may be counted, which is equal to *T<sub>M</sub>/T<sub>C</sub>*; because *T<sub>c</sub>* is known, *T<sub>M</sub>* may be calculated. The time from flash-labeling to the point at which the curve passes the 50% level in Figure 21.5 is *T<sub>0</sub>*? + .5 **TMJ** because *TM* already is known, *T<sub>G2</sub>* may be calculated. The remaining quantity, *T<sub>GI</sub>*, is deduced by subtraction, because the total cycle time and all other phases are known.

A careful examination of an actual set of experimental data makes it plain that the first wave in the percent-labeled mitoses curve is not symmetric; the downswing is shallower than the upswing. This observation, coupled with the fact that the second peak is much smaller than the first, indicates that the population is made up of cells with a wide range of cycle times. In many instances, particularly if the population of cells involved is an *in vivo* specimen of a tumor or a normal tissue, the spread of cell-cycle times is so great that a second peak is barely discernible.

In practice, therefore, the constituent parts of the cycle are not simply read off of the percent-labeled mitoses curve. Instead, a complex computer program is used to try various distributions of cell-cycle times and to calculate the curve that best fits the experimental data. In this way an estimate is obtained of the mean cell-cycle time and also of the range of cell-cycle times in the population.

### EXPERIMENTAL MEASUREMENTS OF CELL-CYCLE TIMES *IN VIVO* *AND IN VITRO*

A vast number of cell-cycle measurements have been made. Only a few representative results are reviewed here to highlight the most important points.

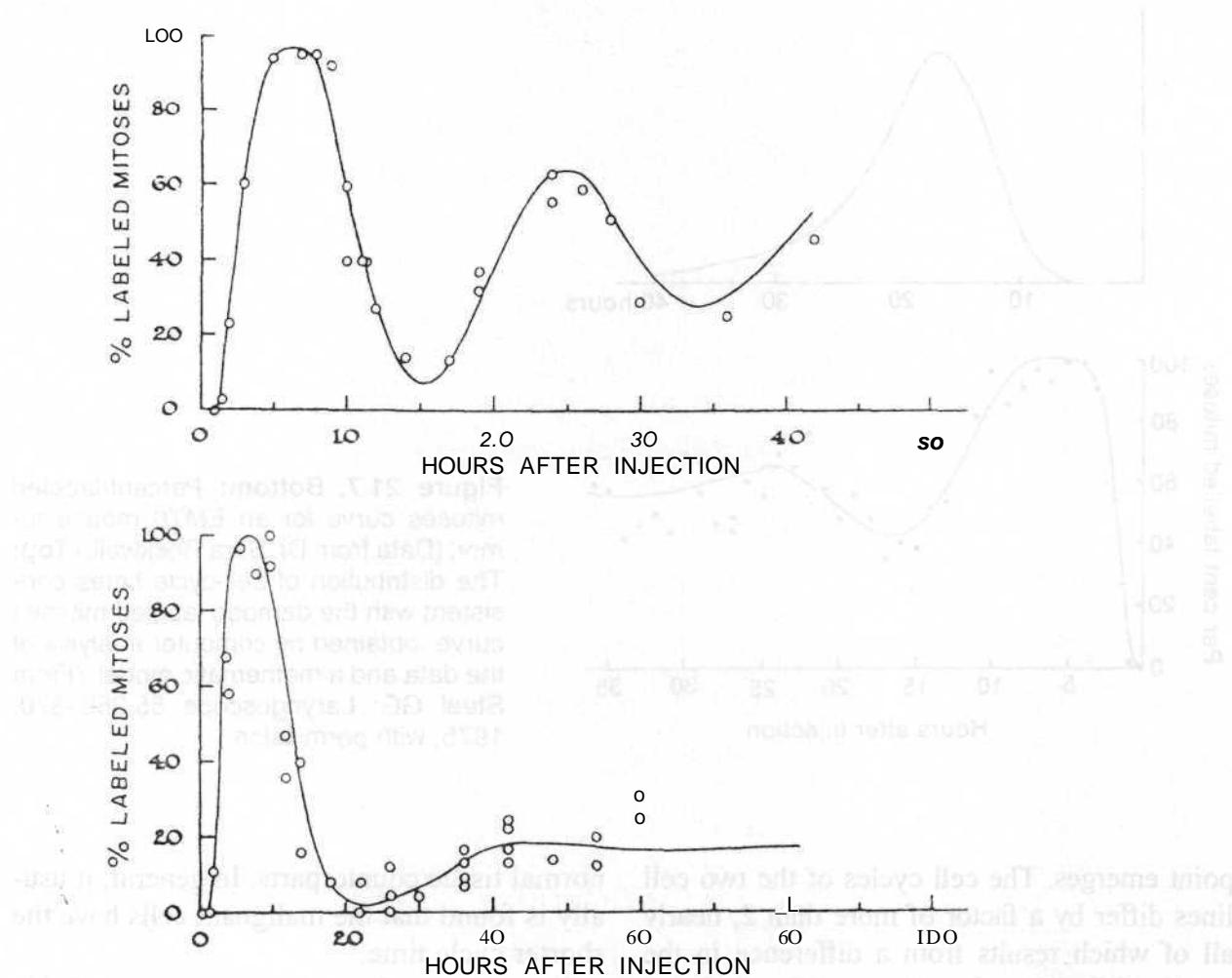
Figure 21.6 shows the percent-labeled mitoses data for two transplantable rat tumors **with** very different growth rates. The tumor represented in the upper panel has a gross doubling time of 22 hours, which can be judged easily from the separation of the first and second waves of labeled mitotic cells. For the tumor illustrated in the lower panel, there is no discernible second peak in the percent-labeled mitoses curve because of the large range of cell cycle times among the cells of the population. To obtain an estimate of the average cell cycle in this case, it is necessary to pool information from the percent-labeled mitoses curve and from a knowledge of the labeling index.

The width of the first wave of the percent-labeled mitoses curve indicates that the length of the DNA synthetic phase (*T<sub>S</sub>*) is about 10 hours. To obtain the average cell-cycle time (*T<sub>c</sub>*), it is essential in this situation to know the labeling index. For this tumor, the labeling index is about 3.6%. The average cell cycle time (*T<sub>C</sub>*) can then be calculated from the following equation:

$$LI = X T_S / T_c$$

Therefore,

$$T_c = 0.693 \times 10 / 3.6/100 = 190 \text{ hours}$$



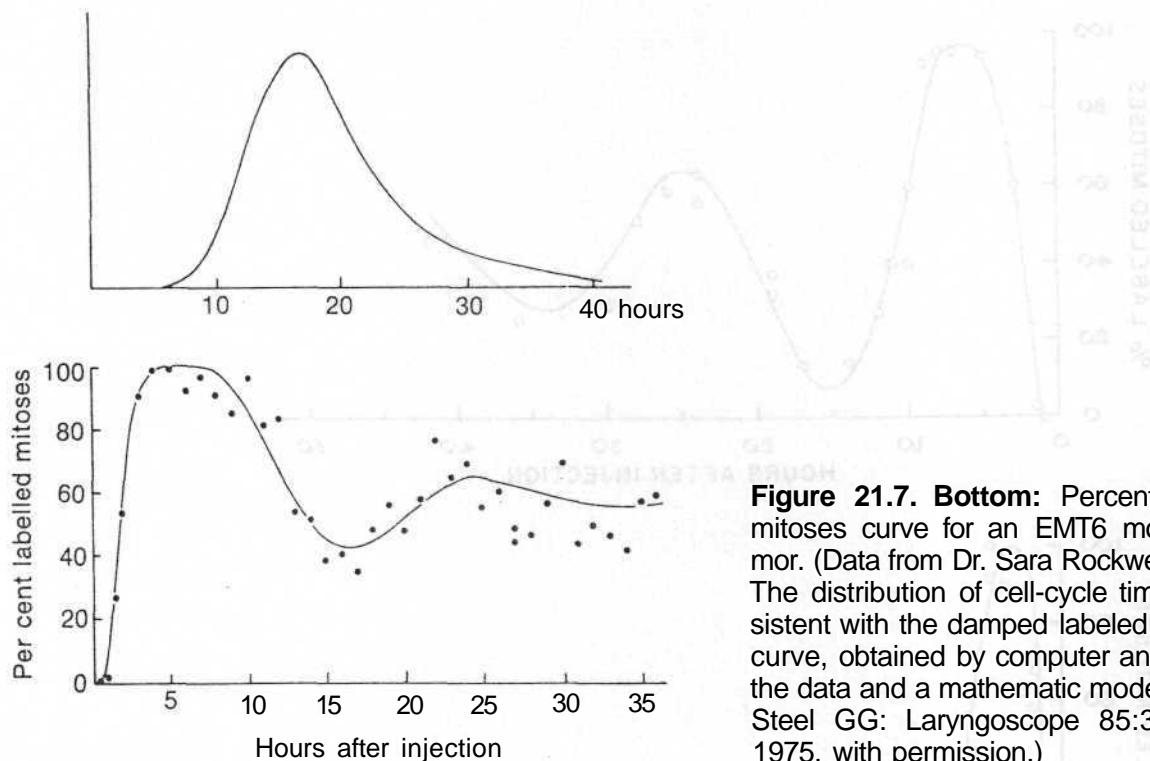
**Figure 21.6.** Percent-labeled mitoses curve for two transplantable rat sarcomas with widely different growth rates. The tumor in the **upper panel** has a gross doubling time of 22 hours, compared with 190 hours for the tumor in the **lower panel**. (From Steel GG, Adams K, Barratt JC: Analysis of the cell population kinetics of transplanted tumours of widely differing growth rate. Br J Cancer 20:784-800, 1966, with permission.)

The absence of a second peak is a clue to the fact that there is a wide range of cell cycle times for the cells of this population, so that 190 hours is very much an average value.

A computer analysis makes\* it possible to estimate the distribution of cell-cycle times in a population. For example, Figure 21.7 shows the percent-labeled mitoses curve for a transplantable mouse tumor, together with an analysis of cell-cycle times based on a mathematical model. There is a wide range of cell-cycle times, from less than 10 to more than 40 hours, with a modal value of about 19 hours.

This range of cycle times explains the damped labeled mitoses curve and the fact that the first peak is not symmetric.

Table 21.1 is a summary of the cell-cycle parameters for cell lines in culture and some of the tissues and tumors for which percent-labeled mitoses curves have been shown in this chapter. The top line of Table 21.1 shows the data for Chinese hamster cells in culture. These cells are characterized by a short cell cycle of only 10 hours and a minimal Gi period. The second row of the table gives the comparable figures for HeLa cells. From a comparison of these two *in vitro* cell lines, a very important



**Figure 21.7. Bottom:** Percent-labeled mitoses curve for an EMT6 mouse tumor. (Data from Dr. Sara Rockwell.) **Top:** The distribution of cell-cycle times consistent with the damped labeled mitoses curve, obtained by computer analysis of the data and a mathematic model. (From Steel GG: Laryngoscope 85:359-370, 1975, with permission.)

point emerges. The cell cycles of the two cell lines differ by a factor of more than 2, nearly all of which results from a difference in the length of Gi. The other phases of the cycle are very similar in the two cell lines.

Also included in Table 21.1 are data for the cells of the normal cheek-pouch epithelium in the hamster and a chemically induced carcinoma in the pouch. These are representative of a number of studies in which cells from a solid tumor have been compared with their

normal tissue counterparts. In general, it usually is found that the malignant cells have the shorter cycle time.

In reviewing the data summarized in Table 21.1, it is at once evident that although the length of the cell cycle varies enormously between populations, particularly *in vivo*, the lengths of G<sub>2</sub>, mitosis, and S are remarkably constant. The vast bulk of the cell-cycle variation is accounted for by differences in the length of the G<sub>i</sub> phase.

**TABLE 21.1. The Constituent Parts of the Cell Cycle for Some Cells in Culture and Tumors in Experimental Animals**

Authors	Cell or Tissue	T <sub>c</sub> , h	T <sub>s</sub> , h	T <sub>M</sub> , h	T <sub>G<sub>2</sub></sub>	T <sub>G<sub>i</sub></sub>
Bedford	Hamster cells <i>in vitro</i>	10	6	1	1	2
	HeLa cells <i>in vitro</i>	23	8	1	3	11
Steel	Mammary tumors in the rat					
	BICR/M1	19	8	-1	2	8
Quastler and Sherman	BICR/A2	63	10	-1	2	50
	Mouse intestinal crypt	18.75	7.5	0.5	0.5-1.0	9.5
	Hamster cheek pouch epithelium	120-152	8.6	1.0	1.9	108-140
Brown and Berry	Chemically induced carcinoma in pouch	10.7	5.9	0.4	1.6	2.8

## PULSED PHOTO CYTOMETRY

During the past several decades, classic autoradiography largely has been replaced by pulsed photo cytometry (Fig. 21.8). The conventional techniques of autoradiography give precise, meaningful answers, but they are laborious and so slow that information is never available quickly enough to act as a predictive assay to influence the treatment options of an individual patient. Techniques based on flow cytometry provide data that are available within a few days. Detailed cell kinetic data can be obtained by such techniques, including an analysis of the distribution of cells in the various phases of the cycle, but in practice the measurement of most immediate **relevance** to clinical radiotherapy is the estimate of  $T_{pot}$ ; the potential tumor doubling time.

## MEASUREMENT OF POTENTIAL TUMOR DOUBLING TIME

$T_{pot}$  is a measure of the rate of increase of cells capable of continued proliferation and therefore may determine the outcome of a ra-

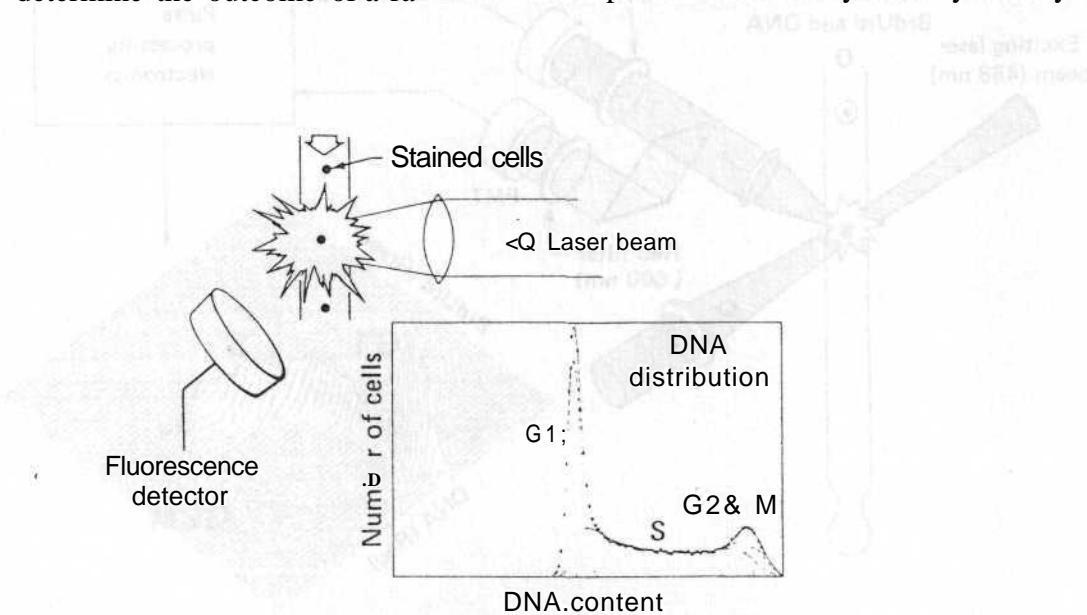
diotherapy treatment protocol delivered in fractions over an extended period of time.

Tumors with a short  $T_{pot}$  may repopulate if fractionation is extended over too long a period.  $T_{pot}$  can be calculated from the following:

$$\bullet \quad T_{pot} = X T_s / L I'$$

in which  $T_s$  is the length of the DNA synthetic period,  $L I$  is the labeling index (*i.e.*, the fraction of cells synthesizing DNA at any time), and  $A$ , is a correction factor to allow for the nonlinear distribution in time of the cells as they pass through the cycle; this factor has a value between 0.67 and 1.

To measure  $T_{pot}$  precisely requires a knowledge of  $T_s$  and the labeling index. The labeling index can be determined from a single sample, but to measure  $T_s$  precisely it is necessary to label the cell population with tritiated thymidine or bromodeoxyuridine, take a sample every hour for a time period about equal to the cell cycle, and count the proportion of labeled mitoses as a function of time, as previously described. This is out of the question in a clinical situation, but an estimate of  $T_{pot}$  can be made by flow cytometry



**Figure 21.8.** The principles of DNA distribution analysis of flow cytometry. Suspensions of fluorescent-stained single cells flow one at a time through a light beam with its wavelength adjusted to excite the fluorescent dye. The fluorescence stimulated in each cell is recorded as a measure of that cell's DNA content. Thousands of cells can be measured each second and the results accumulated to form a DNA distribution like that shown for asynchronously growing Chinese hamster ovary cells. (From Gray JW, Dolbeare F, Pallavicini MG, Beisker W, Waldman F: Cell cycle analysis using flow cytometry. Int J Radiat Biol 49:237-255, 1986, with permission.)

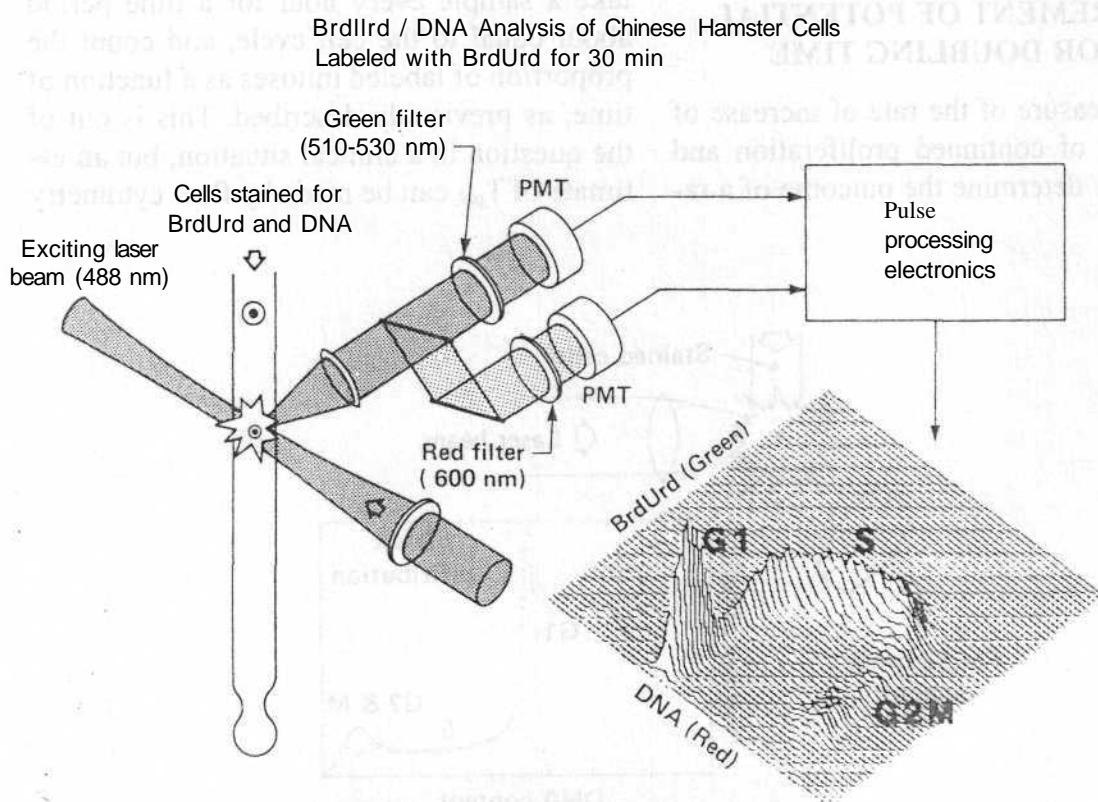
from a single biopsy specimen taken 4 to 8 hours after the injection of a tracer amount of a thymidine analogue (bromodeoxyuridine or iododeoxyuridine). The biopsy specimen is treated with fluorescent-labeled monoclonal antibody, which detects the incorporation of the thymidine analogue into the DNA.

The specimen also is stained with propidium iodide to determine DNA content. A single-cell suspension of the biopsy specimen then is passed through a flow cytometer, which simultaneously measures DNA content (red) and bromodeoxyuridine content (green). This is illustrated in Figure 21.9.

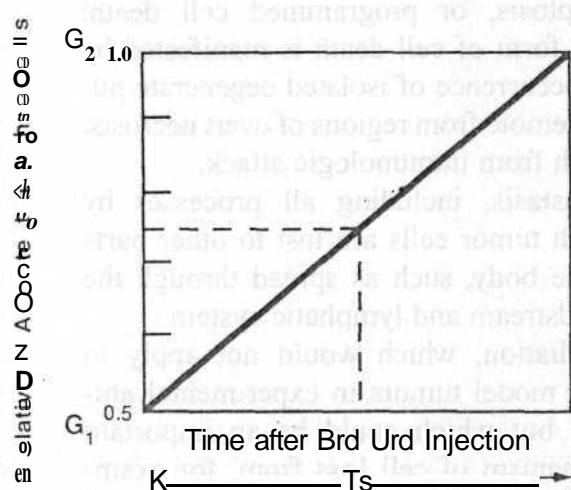
The labeling index is simply the proportion of cells that show significant green fluorescence. Ts can be calculated from the mean red fluorescence of S cells relative to Gi and G2 cells. The DNA content of cells in G2 is dou-

ble that in Gi. The method assumes that the red fluorescence of bromodeoxyuridine-labeled cells (*i.e.*, the DNA content of cells in S phase) increases linearly with time (Fig. 21.10). If, for example, the biopsy specimen were obtained 6 hours after administration of bromodeoxyuridine, and the relative DNA content of cells labeled with bromodeoxyuridine (*i.e.*, in S phase) were 0.75, well between that characteristic of Gi and that of G2, Ts would be simply 12 hours. This method has been validated in a number of *in vitro* cell lines and also in animal tumor systems, in which it can be checked by conventional cell "kinetic studies."

This technique gives an average value for T<sub>pot</sub> of the cells in the biopsy specimen, because the cells are disaggregated and made into single-cell suspensions. There is some ev-



**Figure 21.9.** The flow cytometric analysis of cellular bromodeoxyuridine (BrdUrd) and DNA content for cells stained with fluorescein (linked to BrdUrd) and propidium iodide (linked to DNA). The cells are processed one at a time through a blue (488-nm) laser beam that excites cellular BrdUrd content, and red fluorescence is recorded as a measure of cellular DNA content. The BrdUrd (green fluorescence) axis in the bivariate is logarithmic, with every seven channels representing a doubling of fluorescence intensity. (From Gray JW, Dolbeare F, Pallavicini MG, Beisker W, Waldman F: Cell cycle analysis using flow cytometry. Int J Radiat Biol 49:237-255, 1986, with permission.)



**Figure 21.10.** Graph illustrating the way in which  $T_s$  can be estimated by flow cytometry on cells from a single tumor biopsy specimen taken 4 to 8 hours after an injection of a thymidine analogue (bromodeoxyuridine or iododeoxyuridine). Cells in S phase are identified by the green fluorescence from an antibody to the thymidine analogue. The relative DNA content is measured by the red fluorescence owing to the incorporated propidium iodide. The DNA content in G<sub>2</sub> cells is double that in G<sub>1</sub>. The length of the DNA synthetic phase ( $T_s$ ) can be estimated by the relative DNA content of the S-phase cells in relation to the time between the injection of the thymidine analogue and the biopsy.

idence from animal experiments that individual cells may have much shorter  $T_{pot}$  values.

This technique to measure  $T_{pot}$  has proven to be practical as a predictive assay and has been used on a large number of patients entered in a clinical trial of altered fractionation patterns by the European Cooperative Radiotherapy Group. This is described in more detail in the chapter on predictive assays (Chapter 23).

## THE GROWTH FRACTION

Central to an appreciation of the pattern of growth of solid tumors is the realization that, at any given moment, not all of the tumor cells that are viable and capable of continued growth actually are proceeding through the cell cycle. The population consists of proliferating (P) cells and quiescent (Q) cells. The growth fraction (GF), a term introduced by Mendelsohn, is defined as the ratio of the number of proliferating cells to the total number of cells (P + Q), or

$$GF = P/P + Q$$

There are various ways to estimate growth fraction. One method consists of injecting tritiated thymidine into an animal with a tumor and then, several cell generations later, preparing an autoradiograph from sections of the tumor. The growth fraction is given by the expression

$$GF = \frac{\text{Fraction of cells labeled}}{\text{Fraction of mitoses labeled}}$$

This method assumes that there are two distinct subpopulations, one growing with a uniform cell cycle, the other not growing at all. Continuous labeling is an alternative way to provide an approximate measure of the proportion of proliferating cells. Tritiated thymidine is infused continuously for a time equal to the cell cycle (minus the length of S phase). The fraction of labeled cells then approximates to the growth fraction. Table 21.2 is a summary of growth fractions measured for a variety of solid tumors in experimental animals, which frequently fall between 30 and 50%, even though the tumors vary widely in

**TABLE 21.2.** Growth Fraction for Some Tumors in Experimental Animals

Tumor	Author	Growth Fraction, %
Primary mammary carcinoma in the mouse (G <sub>3</sub> H)	Mendelsohn	35-77
Transplantable sarcoma in the rat (RIB <sub>5</sub> )	Denekamp	55
Transplantable sarcoma in the rat (SSO)	Denekamp	47
Transplantable sarcoma in the rat (SSB1)	Denekamp	39
Mammary carcinoma in the mouse (C3H)	Denekamp	30
Chemically induced carcinoma in the hamster cheek pouch	Brown	29

degree of differentiation, arise in different species, and are of varied histologic types. As a tumor outgrows its blood supply, areas of necrosis often develop that are accompanied by the presence of hypoxic cells, the proportion of which for many solid tumors is about 15%. This accounts for part but not all of the quiescent cell population.

### CELL LOSS

The overall growth of a tumor is the result of a balance achieved between cell production from division and various types of cell loss. In most cases, tumors grow much more slowly than would be predicted from a knowledge of the cycle time of the individual cells and the growth fraction. The difference is a result of cell loss. The extent of the cell loss from a tumor is estimated by comparing the rate of production of new cells with the observed growth rate of the tumor. The discrepancy provides a measure of the rate of cell loss. If  $T_{pot}$  is the potential tumor-doubling time, calculated from the cell cycle time and the growth fraction, and  $T_d$  is the actual tumor-doubling time, obtained from simple direct measurements on the diameter of the tumor mass, the cell-loss factor ( $\Phi$ ) has been defined by Steel to be

$$\Phi = 1 - T_{pot}/T_d$$

The cell-loss factor represents the *ratio of the rate of cell loss to the rate of new cell production*. It expresses the loss of growth potential by the tumor. A cell-loss factor of 100%, for instance, indicates a steady state of neither growth nor regression.

Cells in tumors can be lost in a number of ways:

- I. Death from inadequate nutrition: As the tumor outgrows its vascular system, rapid cell proliferation near capillaries pushes other cells into regions remote from blood supply, in which there is an inadequate concentration of oxygen and other nutrients. These cells die, giving rise to a progressively enlarging necrotic zone.

2. Apoptosis, or programmed cell death: This form of cell death is manifested by the occurrence of isolated degenerate nuclei remote from regions of overt necrosis.
3. Death from immunologic attack.
4. Metastasis, including all processes by which "tumor" cells are lost to other parts of the body, such as spread through the bloodstream and lymphatic system.
5. Exfoliation, which would not apply to most model tumors in experimental animals but which could be an important mechanism of cell loss from, for example, carcinoma of the gastrointestinal tract, in which the epithelium is renewed at a considerable rate.

There are limited data on the relative importance of these different processes in different tumor types, but it is clear that death from inadequate nutrition—by entry into necrotic areas—is often a major factor. It reflects the latent inability of the vascular system to keep up with the rate of cell production. There is still a great deal to be learned about the occurrence of cell loss from tumors, the mechanisms by which it occurs, and the factors by which it can be controlled. It is clear, however, that any understanding of the growth rate of tumors at the cellular level must include a consideration of this often dominant factor.

### DETERMINATIONS OF CELL LOSS IN EXPERIMENTAL ANIMAL TUMORS

The cell-loss factor has been estimated in a considerable number of tumors in experimental animals. Some of the results are listed in Table 21.3. Values for the cell-loss factor vary from 0 to more than 90%. In reviewing the literature on this subject, Denekamp pointed out that sarcomas tended to have low cell-loss factors, but carcinomas tended to have high cell-loss factors. All of the sarcomas investigated had cell-loss factors less than 30%; the carcinomas had cell-loss factors in excess of 70%. Therefore, cell loss appears to be a dominant factor in the growth of carcinomas and of considerably less importance for sarcomas. This pattern correlates with the importance of

**TABLE 21.3.** The Cell Loss Factor ( $\delta$ ) for Some Tumors in Experimental Animals

Tumor	Author	$\delta$ , %
Mouse sarcoma	Frindel	0
3-day-old tumor		10
7-day-old tumor		55
20-day-old tumor		
Rat carcinoma	Steel	9
Rat sarcoma	Steel	0
Mouse carcinoma	Mendelsohn	69
Hamster carcinoma	Brown	75
Rat sarcoma	Hermens	26
Hamster carcinoma	Reiskin	81-93
Mouse carcinoma	Tannock	70-92

apoptosis as a mode of cell death. Apoptosis is quite common in carcinomas and rare in sarcomas.

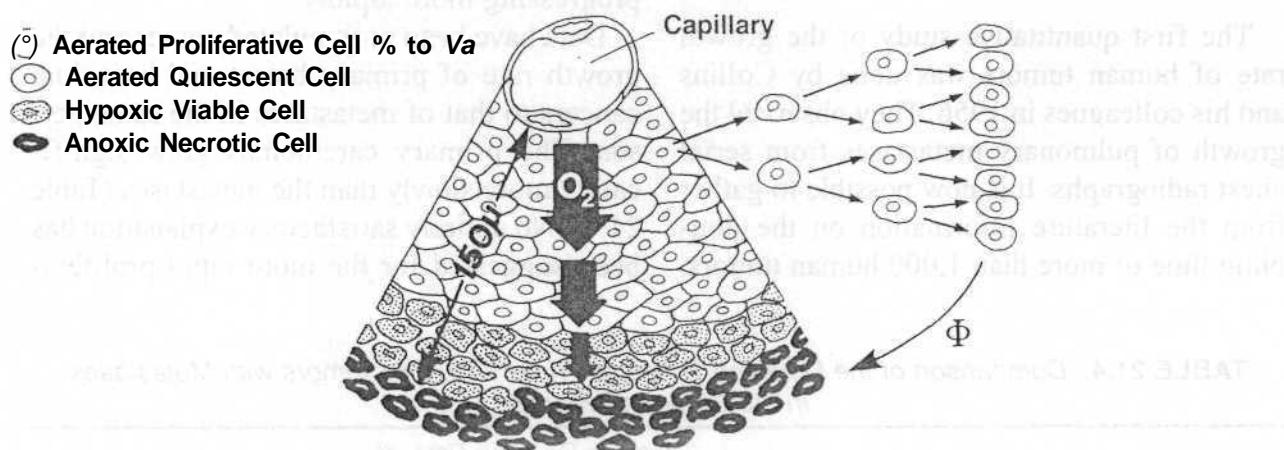
If this is found to be a general phenomenon, it might be attributed to the origin of carcinomas from continuously renewing epithelial tissues, in which the cell-loss factor is 100%. This difference between sarcomas and carcinomas also may account for their differing responses to radiation. In carcinomas, in which the production of new cells is temporarily stopped or reduced by a dose of radiation, cells continue to be removed from the tumor because of the high cell-loss factor, and

the tumor shrinks. In sarcomas, however, even if a large proportion of the cells are sterilized by a dose of radiation, they do not disappear from the tumor mass as quickly.

It would be simple, then, to explain why two tumors, one a carcinoma and one a sarcoma, containing the same number of cells and exposed to the same radiation dose, would appear to behave quite differently. The carcinoma might shrink dramatically soon after the radiation dose, whereas the sarcoma would not appear as affected by the radiation. In the long term, the "cure" rates of both tumors may well be identical, but in the short term the carcinoma would be said to have "responded" to the radiation, whereas the sarcoma might be said to be unresponsive or resistant to radiation.

### THE OVERALL PATTERN OF TUMOR GROWTH

Three factors determine the growth rate of a tumor: (1) the cell cycle of the proliferative cells in the population; (2) the growth factor, that fraction of cells in the population that is proliferating as opposed to quiescent; and (3) the rate of cell loss, either by cell death or loss from the tumor (Fig. 21.11).



**Figure 21.11.** The overall pattern of the growth of a tumor. Clonogenic cells consist of proliferative (P) and quiescent (Q) cells. Quiescent cells can be recruited into the cell cycle as the tumor shrinks after treatment with radiation or a cytotoxic drug. In animal tumors the growth fraction is frequently 30 to 50%. Of the cells produced by division, many are lost, principally into necrotic areas of the tumor remote from the vasculature. The cell loss factor ( $\delta$ ) varies from 0 to 100% and dominates the pattern of tumor growth. As the tumor outgrows its blood supply, some cells become hypoxic. This accounts for some of the quiescent cells that are out of cycle.

The proliferative cells of a tumor, unrestrained as they are by any homeostatic control, divide and proliferate as rapidly as they are able to, limited only by their own inherited characteristics and the availability of an adequate supply of nutrients. Because a tumor is not an organized tissue, it tends to outgrow its blood supply. Areas of necrosis often develop and frequently are accompanied by hypoxic cells, which often constitute about 15% of the total viable cells.

Another manifestation of the overstretched vascular supply is that only a proportion of the viable cells (the growth fraction) actually are proceeding **through** the cell cycle and multiplying. The growth fraction is frequently 30 to 50% but tends to be higher in regions close to blood capillaries and lower near necrotic areas.

The potentially explosive growth rate of tumors seldom is realized in practice because of cell loss from metastasis, exfoliation, random cell death, or cell death in the necrotic areas of the tumor. The cell-loss factor (the fraction of cells produced by mitosis that is lost) is the most variable factor observed among tumors in experimental animals: Values from 0 to more than 90% have been reported.

### GROWTH KINETICS OF HUMAN TUMORS

The first quantitative study of the growth rate of human tumors was done by Collins and his colleagues in 1956. They observed the growth of pulmonary metastases from serial chest radiographs. It is now possible to gather from the literature information on the doubling time of more than 1,000 human tumors.

Most of the data were obtained either by measurements from radiographs or by direct measurements of skin tumors or metastases in soft tissue. The doubling time of human tumors varies widely from patient to patient and is on the average very long; Tubiana and Malaise have estimated that the median value is about 2 months.

Tumors of the same histologic type arising in different patients differ widely in growth rate. By contrast, metastases arising in the same patient tend to have similar rates of growth. The latter observation is the basis for using patients with multiple skin or pulmonary metastases to test and compare new treatment modalities, such as high linear energy transfer radiations or hyperthermia. There is certainly a correlation between histologic type and growth rate. Tubiana and Malaise have collected values for the doubling time in 389 patients with pulmonary metastases, classified into five histologic categories. They can be arranged in order of doubling time as follows: embryonic tumors, 27 days; malignant lymphomas, 29 days; mesenchymal sarcomas, 41 days; squamous cell carcinomas, 58 days; and adenocarcinomas, 82 days. In addition, the degree of differentiation seems to be related to the doubling time, with poorly differentiated cancers generally progressing more rapidly.

Data have been accumulated comparing the growth rate of primary breast and bronchial cancers to that of metastases in the same person. The primary carcinomas grew significantly more slowly than the metastases (Table 21.4). No entirely satisfactory explanation has been suggested for the more rapid prolifera-

TABLE 21.4. Comparison of the Doubling Times of Primary Human Tumors with Metastases in the Same Individuals

Histologic Type	Average Doubling Time, d <sup>a</sup>	
	Primary Tumors	Lung Metastases
Squamous cell carcinomas	81.8(97)	58.0 (51)
Adenocarcinomas	166.3 (34)	82.7 (134)

<sup>a</sup>Number of patients in parentheses.

From Charbit A, Malaise E, Tubiana M: Eur J Cancer 7:307, 1971, with permission.

tion of metastases. It may be a question of selection or a function of the unusually favorable milieu into which secondary tumors tend to be seeded.

In addition to growth-rate measurements, studies of cell population kinetics also have been performed on a limited number of human tumors. Studies of this kind raise practical and ethical problems. The ethical problems stem from the fact that *in vivo* experiments require an injection of tritiated thymidine or bromodeoxyuridine, which limits such studies to patients who have short life expectancies and in whom the injection of the label does not raise any problems of possible genetic consequences. The practical problems arise because the percent-labeled mitoses technique, which is the most satisfactory way to obtain the duration of the various phases of the cell cycle, requires a large number of sequential samples to be taken for several days after the injection of the label. In mice or rats the multiple samples are obtained from a large number of identical animals, bearing transplanted tumors of the same size and type, by sacrificing one or more animals each time. In humans each spontaneous tumor is unique, so the multiple samples must be obtained by repeated biopsies at frequent intervals from the same tumor. This heroic procedure is uncomfortable and inconvenient for the patient and is only practical with large superficial skin tumors, which may not be truly representative of human tumors in general. Nevertheless, a surprisingly large number of human tumors have been studied in this way. The percent-labeled mitoses curves obtained are similar to those for laboratory animals, although in general the second wave of labeled mitoses is rarely distinct and is usually altogether absent.

Tubiana and Malaise surveyed the field and reported 41 cases in which the cell cycle of solid tumors in humans had been evaluated with the percent-labeled mitoses technique. The cell cycles observed were between 15 and 125 hours in 90% of the cases, with a modal value of 48 hours (Table 21.5). The

duration of S ( $T_s$ ) was less variable than the total cell cycle, with 90% of the values falling between 9.5 and 24 hours and a modal value of about 16 hours. As a first approximation, it can be assumed that  $T_s$  has a duration of about 16 hours and that the mean duration of the cell cycle is about three times the duration of  $T_s$ .

Although the percent-labeled mitoses technique has been used on relatively few human tumors, the labeling index has been measured in many more after *in vitro* incubation of a fresh piece of excised tumor with tritiated thymidine. The rationale underlying this technique is that cells already synthesizing DNA *in vivo* are able to continue synthesis of DNA *in vitro*, but no new cells enter synthesis under the incubation conditions that normally are used.

The growth fraction, too, has been measured in only a limited number of human tumors by the method of continuous labeling. If

TABLE 21.5. Individual Values for the Duration of the Cell Cycle ( $T_c$ ) of Human and Solid Tumors of Various Histologic Types

Authors	$T_c$ , h
Frindel et al. (1968)	97, 51.5, 27.5, 48, 49.8
Bennington (1969)	15.5, 14.9
Young and de Vita (1970)	42, 82, 74
Shirakawa et al. (1970)	120, 144
Weinstein and Frost (1970)	217
Terz et al. (1971)	44.5, 31, 14, 25.5, 26,
Peckham and Steel (1973)	59
Estevez et al. (1972)	37, 30, 48, 30, 38, 96, 48
Terz and Curutchet (1974) <sup>a</sup>	18, 19, 19.2, 120
Malaise et al. (unpublished data) <sup>3</sup>	24, 33, 48, 42
Muggia et al. (1972)	64
Bresciani et al. (1974)	82, 50, 67, 53, 58

<sup>a</sup>Measured by the mean grain count halving time.

From Tubiana M, Malaise E: Growth rate and cell kinetics in human tumors: Some prognostic and therapeutic implications. In Symington T, Carter RL (eds): Scientific Foundations of Oncology, pp 126-136. Chicago, Year Book Medical Publishers, 1976, with permission.

a population of cells is labeled continuously during a period corresponding to the duration of the cell cycle less the duration of the DNA synthetic phase (*i.e.*,  $T_c - T_s$ ), all the actively proliferating cells should be labeled. This method of continuous labeling can be performed only with a small number of patients who are in no way representative. An alternative procedure is to estimate the growth fraction by assuming that the proportion of cells in cycle is about equal to three times the labeling index, an assumption based on the notion that the cell cycle is three times the length of the S phase. The growth fraction calculated in this way correlates well with the tumor-doubling time: It is 0.9 in malignant lymphomas and embryonic tumors and less than 0.06 in adenocarcinomas. The relation between the growth rate and growth fraction appears to be much closer in human tumors than in animal tumors.

Of the various parameters that characterize tumor kinetics, the cell-loss factor is, in general, the most difficult to evaluate. The cell-loss factor for human tumors generally has been calculated by comparing the *observed tumor-volume doubling time* with the *potential doubling time*, which is the time required for the population of cells to double, assuming that all the cells produced are retained in the tumor. Tubiana and Malaise calculated a mean value of the cell loss factor for five histologic groups of human tumors, assuming the duration of S to be 16 hours. Their results suggest that, in general, the mean cell-loss factor exceeds 50%. Furthermore, it appeared

to be higher if the tumor was growing quickly and if its growth fraction was high. In humans, the smallest cell-loss factors seem to be associated with those histologic types of tumors that have the slowest rate of growth. The cell-loss factor, therefore, tends to reduce the spread of growth rates that results from the differences in growth fraction of the various types of tumors.

Steel has estimated independently the extent of cell loss in human tumors by comparing the potential doubling time with observed tumor-growth rates. The relevant data on the volume-doubling time for six groups of human tumors are shown in Table 21.6. They consist mostly of measurements of primary and secondary tumors of the lung. There are differences between individual series, which indeed may reflect significant differences in the growth rates of the various types of tumors, but if the results are all pooled, they yield an average median doubling time of 66 days, with 80% of the values falling in the range between 18 and 200 days. Taking the median values for the labeling index, doubling time, and S phase as suggested by Steel, the median cell loss factor in all human tumors studied is 77%. It thus would appear that for human tumors, cell loss is generally the most important factor determining the pattern of tumor growth.

The high rate of cell loss in human tumors largely accounts for the great disparity between the cell-cycle time of the individual dividing cells and the overall doubling time of the tumor. Although the tumor doubling time

TABLE 21.6. Volume-Doubling Times of Human Tumors

Authors	Site	Volume-doubling Time, d	Range, d
Breuer	Lung metastases	40	4-745
Collins et al.	Lung metastases	40	11-164
Collins	Lung metastases from colon or rectum	96	34-210
Garland	Primary bronchial carcinoma	105	27-480
Schwartz	Primary bronchial carcinomas	62	17-200
Spratt	Primary skeletal sarcomas	75	21-366

Based on data from Steel GG: Cell loss from experimental tumours. Cell Tissue Kinet 1:193-207, 1968.

is characteristically 40 to 100 days, the cell-cycle time is relatively short, 1 to 5 days. This has important implications, which often are overlooked, in the use of cycle-specific chemotherapeutic agents or radiosensitizing drugs, for which it is the cell-cycle time that is relevant.

Because Bergonié and Tribondeau established a relation between the rate of cell proliferation and the response to irradiation in normal tissues, it might be supposed that this would be the same for tumors. It is of interest to note that the histologic groups of human tumors that have the most rapid mean growth rates and the highest growth fractions and cell-turnover rates are indeed those that are the most radiosensitive. There is also a correlation between, on the one hand, the growth rate and the labeling index or the cell loss and, on the other hand, the reaction to chemotherapy. This is not surprising, because the majority of drugs act essentially on cells in S phase. It is remarkable, however, that the only human tumors in which it is possible to achieve cures by chemotherapy are the histologic types with high labeling indexes. Furthermore, a high level of cell loss appears to favor the response to chemotherapy, and in humans this occurs especially in tumors with high labeling indexes.

### COMPARISON OF THE CELL CYCLE TIMES OF THE CELLS OF SOLID TUMORS AND THEIR NORMAL COUNTERPARTS

A number of authors has attempted to compare the cell-cycle times of normal and malignant tissues. Despite the paucity of data available, it is fair to draw the general conclusion that the cell-cycle time of the malignant cells is appreciably less than that of their normal counterparts. An exception to this generalization is tumors arising from rapidly proliferating normal tissues, such as leukemias and tumors of the gastrointestinal tract. In these cases it is unlikely that the tumor cells have a

shortened cell cycle *compared with the comparable normal tissue.*

It generally is found that irradiation causes an *elongation* of the generation cycle of tumor cells; a corresponding *shortening* of the cell cycle of normal cells is frequently reported. There are exceptions to this rule, but it often is found to be true.

From investigations of the morphologic and proliferative changes produced by radiation in the epidermis of hairless mice, Devi showed that the cell-cycle time of the basal cells of this epidermis was reduced dramatically compared with unirradiated cells, and that this reduction occurred in regions showing a gross hyperplastic reaction after irradiation. A similar dramatic shortening of the cell cycle of irradiated epidermal cells is suggested by data reported by Breuer from patients who had just completed a course of radiotherapy, after which the skin overlying the treated region had undergone moist desquamation.

It would seem, therefore, that the cycle time of cells with a long generation time can be shortened substantially by irradiation. Such results would appear, however, to depend on a gross tissue response. If the turnover time, or the cell-cycle time, of a tissue is very long, the tissue takes a long time to recognize the fact that most of its cells have been sterilized by a dose of radiation, because cells only die in attempting a subsequent mitosis.

The probable consequence of this is that normal tissues do not respond by speeding up the progress of cells through their cell cycle for some time after the delivery of the dose of radiation. If this time is comparable to the overall time of a fractionated course of radiotherapy, then the preirradiation cell-cycle times of the normal and malignant tissues are relevant to a discussion of dose-time relationships in radiotherapy and to the use of cycle-specific chemotherapeutic or radiosensitizing drugs in combination with radiotherapy.

### SUMMARY OF PERTINENT CONCLUSIONS

- Mammalian cells proliferate by mitosis (M). The interval between successive mitoses is the cell cycle time ( $T_c$ ).
- In mitosis the chromosomes condense and are visible. The DNA synthetic (S) phase can be identified by autoradiography. The first gap ( $G_1$ ) separates mitosis from S phase. The second gap ( $G_2$ ) separates S phase from the subsequent mitosis.
- Fast-growing cells in culture and some cells in self-renewal tissues *in vivo* have  $T_{fcs}$  of 10 hours; stem cells in a resting normal tissue, such as the skin, have a cell cycle time of 10 days.
- Most of the difference in cell cycle between fast- and slow-growing cells is a result of differences in  $G_1$ , which varies from less than 1 hour to more than a week.
- The mitotic index (MI) is the fraction of cells in mitosis:

$$MI = XT_u/T_c$$

- The labeling index (LI) is the fraction of cells that take up tritiated thymidine (i.e., the fraction of cells in S):

$$LI = XT_s/T_c$$

- The percent-labeled mitoses technique allows an estimate to be made of the lengths of the constituent phases of the cell cycle. The basis of the technique is to label cells with tritiated thymidine or bromodeoxyuridine in S phase and time their arrival in mitosis.
- Flow cytometry allows a rapid analysis of the distribution of cells in the cycle. Cells are stained with a DNA-specific dye and sorted on the basis of DNA content.
- The bromodeoxyuridine-DNA assay in flow cytometry allows cells to be stained simultaneously with two dyes that fluoresce at different wavelengths: One binds in proportion to DNA content to indicate the phase of the cell cycle and the other binds in proportion to bromodeoxyuridine incorporation to show if cells are synthesizing DNA.
- $T_{pot}$  the potential doubling time of a tumor, reflects the cell cycle of individual cells and the growth fraction but ignores cell loss.
- $T_{pot}$  may be the relevant parameter for estimating the effect of cell proliferation on a protracted radiotherapy protocol.
- $T_{pot}$  can be estimated by means of flow cytometric analysis on cells from a single biopsy specimen taken 4 to 8 hours after an intravenous administration of bromodeoxyuridine.
- The growth fraction is the fraction of cells in active cell cycle (i.e., the fraction of proliferative cells).
- In animal tumors, the growth fraction frequently ranges from 30 to 50%.
- The cell-loss factor (\$) is the fraction of cells produced by cell division lost from the tumor.
- In animal tumors,  $\langle \$ \rangle$  varies from 0 to more than 90%, tending to be small in small tumors and to increase with tumor size.
- The # tends to be large for carcinomas and small for sarcomas.
- Volume-doubling time of a tumor is the gross time for it to double overall in size as measured, for example, in serial radiographs.
- $T_{pot}$  is the calculated time for a tumor to double in size, allowing for the growth fraction but assuming that all cells produced are retained in the tumor.
- Tumors grow much more slowly than would be predicted from the cycle time of individual cells. One reason is the growth fraction, but the principal reason is the cell-loss factor.

- The overall pattern of tumor growth may be summarized as follows. A minority of cells (the growth fraction) is proliferating rapidly; most are quiescent. The majority of the new cells produced by mitosis is lost from the tumor.
- In general, the cell-cycle time of malignant cells is appreciably shorter than that of their normal tissue counterparts.
- In general, irradiation causes an elongation of the cell-cycle time in tumor cells and a shortening of the cell cycle in normal tissues.
- In 90% of human tumors the cell cycle time is between 15 and 125 hours (modal value, 48 hours).
- In human tumors,  $T_s$  has a modal value of about 16 hours (a range of 9.5-24 hours).
- As a first approximation, the mean duration of the cell cycle in human tumors is about three times the duration of the S phase.
- Growth fraction is more variable in human tumors than in rodent tumors and correlates better with gross volume-doubling time.
- Cell-loss factor for human tumors has been estimated by Tubiana and Malaise to have an average value for a range of tumors in excess of 50%. Steel's estimate for a median value for all human tumors studied is 77%.

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## 22

# Time, Dose, and Fractionation in Radiotherapy

THE INTRODUCTION OF FRACTIONATION  
THE FOUR Rs OF RADIobiOLOGY  
THE STRANDQUIST PLOT AND THE ELLIS NOMINAL STANDARD DOSE SYSTEM  
PROLIFERATION AS A FACTOR IN NORMAL TISSUES  
THE SHAPE OF THE DOSE-RESPONSE RELATIONSHIP FOR EARLY-AND LATE-RESPONDING TISSUES  
POSSIBLE EXPLANATIONS FOR THE DIFFERENCE IN SHAPE OF DOSE-RESPONSE RELATIONSHIPS FOR EARLY- AND LATE-RESPONDING TISSUES

FRACTION SIZE AND OVERALL TREATMENT TIME: INFLUENCE ON EARLY- AND LATE-RESPONDING TISSUES  
ACCELERATED REPOPULATION MULTIPLE FRACTIONS PER DAY LESSONS LEARNED FROM FRACTIONATION STUDIES USING THE LINEAR-QUADRATIC CONCEPT TO CALCULATE EFFECTIVE DOSES IN RADIOTHERAPY SUMMARY OF PERTINENT CONCLUSIONS

### THE INTRODUCTION OF FRACTIONATION

The multifraction regimens commonly used in conventional radiation therapy are a consequence largely of radiobiologic experiments performed in France in the 1920s and 1930s. It was found that a ram could not be sterilized by exposing its testes to a single dose of radiation without extensive skin damage to the scrotum, whereas if the radiation was spread out over a period of weeks in a series of daily fractions, sterilization was possible without producing unacceptable skin damage (Fig. 22.1). It was postulated that the testes were a model of a growing tumor, whereas the skin of the scrotum represented a **dose-limiting** normal tissue. The reasoning may be flawed, but the conclusion proved to be valid: Fractionation of the radiation dose produces, in most cases, better

tumor control for a given level of normal tissue toxicity than a single large dose.

### THE FOUR Rs OF RADIobiOLOGY

Now, more than 70 years later, we can account for the efficacy of fractionation based on more relevant radiobiological experiments. We can appeal to the four Rs of radiobiology:

Repair of sublethal damage  
Reassortment of cells within the cell cycle  
Repopulation  
Reoxygenation

The basis of fractionation in radiotherapy can be understood in simple terms. Dividing a dose into a number of fractions spares normal tissues because of repair of sublethal damage between dose fractions and repopulation of



**Figure 22.1.** Conventional multifraction radiotherapy was based on experiments performed in Paris in the 1920s and 1930s. Rams could not be sterilized with a single dose of x-rays without extensive skin damage, whereas if the radiation were delivered in daily fractions over a period of time, sterilization was possible without skin damage. The testes were regarded as a model of a growing tumor and skin as dose-limiting normal tissue.

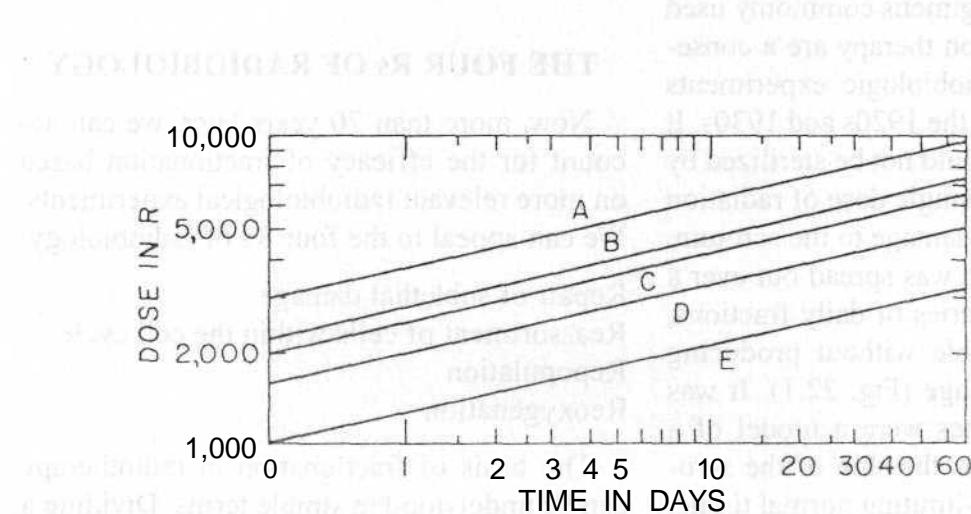
cells if the overall time is sufficiently long. At the same time, dividing a dose into a number of fractions increases damage to the tumor because of reoxygenation and reassortment of cells into radiosensitive phases of the cycle between dose fractions.

The advantages of prolongation of treatment are to spare early reactions and to allow adequate reoxygenation in tumors. Excessive prolongation, however, allows surviving tumor cells to proliferate during treatment.

### THE STRANDQUIST PLOT AND THE ELLIS NOMINAL STANDARD DOSE SYSTEM

Early attempts to understand and account for fractionation gave rise to the well-known Strandquist plot, in which effective single dose was plotted as a function of the overall treatment time (Fig. 22.2). Because all treatments were given as three or five fractions per week, overall time in this plot contains by implication the number of fractions as well. It commonly was found in these plots that the slope of the isoeffect curve for skin was about 0.33; that is, the total dose for an isoeffect was proportional to  $T^{0.33}$ .

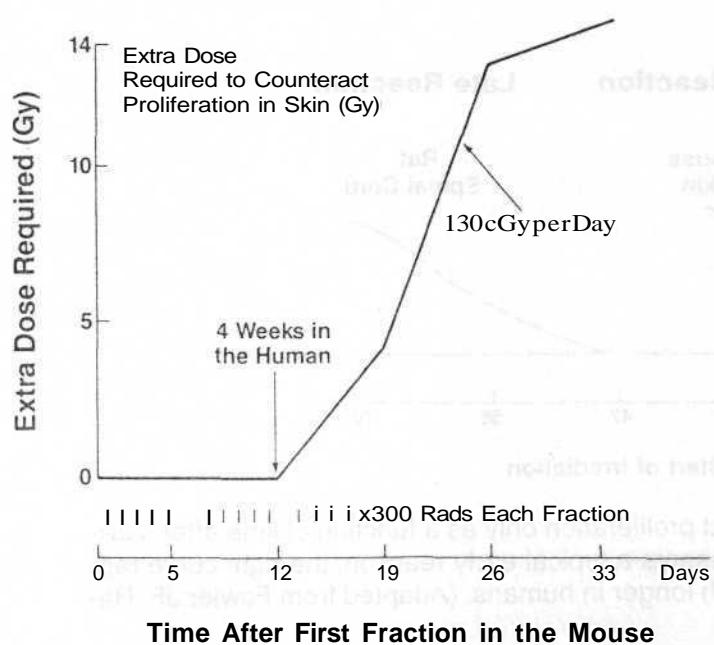
The most important contribution in this area, made by Ellis and his colleagues with the introduction of the nominal standard dose (NSD) system, was the recognition of the importance of separating overall time from the number of fractions. According to this hypothesis, total dose for the tolerance of connective tissue is related to the number of fractions (N) and the overall time (T) by the relation:



**Figure 22.2.** Isoeffect curves relating the total dose to the overall treatment time for skin necrosis (A), cure of skin carcinoma (B), moist desquamation of skin (C), dry desquamation of skin (D), and skin erythema (E). (Adapted from Strandquist M: Acta Radiol 55[suppl]:1-30O, 1944, with permission.)

$$\text{Total dose} = (\text{NSD})T^{0.11}N^{0.24}$$

The NSD system has been discussed extensively. It does enable predictions to be made of equivalent dose regimens, provided that the range of time and number of fractions are not too great and do not exceed the range over which the data are available. For example, in changing a treatment protocol from five to four fractions per week, the formula can be used to calculate the size of dose fractions needed to result in the same normal tissue tolerance with the two different protocols. Of course, because the system is based ultimately on skin-reaction data, it does not in any way predict *late effects*. An obvious weakness of the NSD system is that time is allowed for in terms of a single power function, in which the nominal single dose is proportional to  $T^{\alpha}$ . In fact, biologic experiments with small animals have shown that this relationship is far from accurate. Proliferation does not affect the total dose required to produce a given biologic reaction at all until some time after the start of irradiation, but then the dependence in time is much greater than allowed for by the Ellis formula.



## PROLIFERATION AS A FACTOR IN NORMAL TISSUES

Experimental evidence indicates that the total dose required to produce a given biologic effect is not a power function of time, as postulated by the Ellis NSD system, but turns out to be more complex. The extra dose required to counter proliferation and result in a given level of skin damage in mice does not increase at all until about 12 days into a fractionated regimen, but then it increases very rapidly as a function of time. The shape of the curve is roughly sigmoidal (Fig. 22.3). If similar data were available for humans, the effects of proliferation would not be seen until a longer period into a fractionation regimen because of the slower response of the human skin and the longer cell cycle of the individual cells. Figure 22.3 is not meant to be quantitative but to indicate that the shape of the curve relating extra dose to proliferation is sigmoidal. This illustrates immediately that the method of allowing for overall time in the NSD system is incorrect or at best a very crude approximation.

A further consideration is that all normal tissues are not the same. In particular, there is

**Figure 22.3.** The extra dose required to counteract proliferation in the skin of mice as a function of time after starting daily irradiation with 300 cGy per fraction. A delay followed by a rapid rise is typical of time factors in proliferating normal tissues. In mouse skin the delay is about 2 weeks; in humans it is about 4 weeks. (Adapted from Fowler JF: Acta Radiol 23:209-216, 1984, with permission; data from Denekamp J: Br J Radiol 46:381, 1973.)

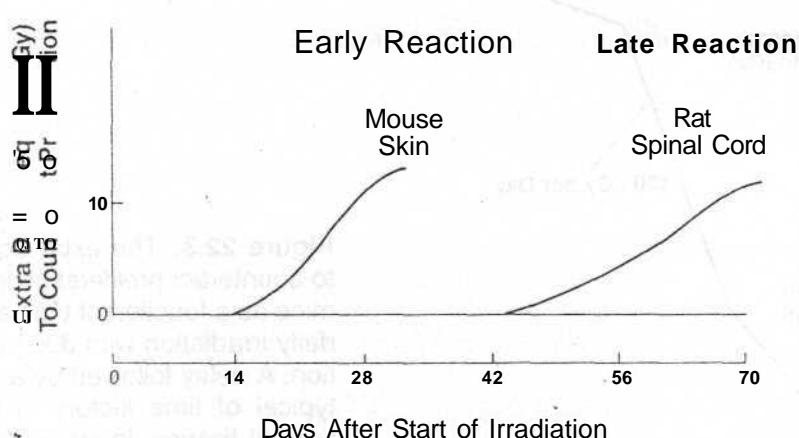
a clear distinction between tissues that are early responding, such as the skin, mucosa, and intestinal epithelium, and those that are late responding, such as the spinal cord. Figure 22.4 shows the extra dose required to produce a given level of damage for a fractionated protracted regimen in the case of representative tissues from the early- and late-responding groups. This diagram compares mouse skin, representative of early-responding tissues, and rat spinal cord, representative of late-responding tissues. It is recognized that these may not be ideal examples, but suitable data for more relevant systems are simply not available; comparable quantitative data certainly are not available for humans. The point made by this figure is that the time after the start of a fractionated regimen at which extra dose is required to compensate for cellular proliferation is quite different for late-, as opposed to early-, responding tissues. The other point made, of course, is that these are data from rodents and that in the case of humans the time scales (although they are not known with any precision) are likely to be very much longer. In particular, the time at which extra dose is required to compensate for proliferation in late-responding tissues in humans is far beyond

the overall time of any normal radiotherapy regimen.

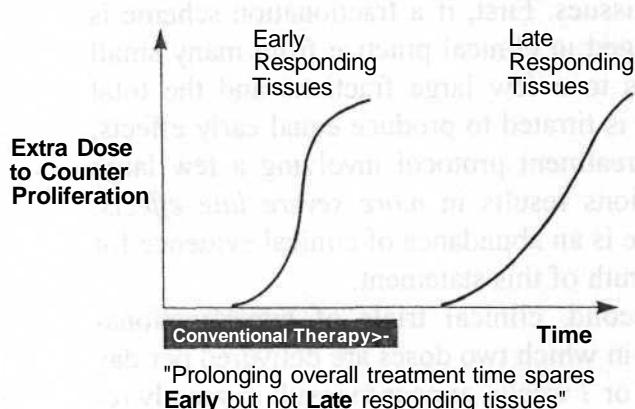
Figure 22.5 is an attempt to convert the experimental laboratory data contained in Figure 22.4 into a general principle that can be applied to clinical practice. Early-responding tissues are triggered to proliferate within a few weeks of the start of a fractionated regimen so that the "extra dose to counter proliferation" increases with time, certainly during conventional radiotherapeutic protocols. By contrast, conventional radiotherapy extending to 6 or 8 weeks is never long enough to allow the triggering of proliferation in late-responding tissues. These considerations lead to the important axiom that

Prolonging overall time within the normal radiotherapy range has little sparing effect on late reactions but a large sparing effect on early reactions.

This has far-reaching consequences in radiotherapy. Early reactions, such as reactions of the skin or of the mucosa, can be dealt with easily by the simple expedient of prolonging the overall time. Although such a strategy overcomes the problem of the early reactions, it has no effect whatsoever on the late reactions.



**Figure 22.4.** The extra dose required to counteract proliferation only as a function of time after starting daily irradiation in rodents. The left curve represents a typical early reaction; the right curve represents a typical late reaction. The delays are much longer in humans. (Adapted from Fowler JF: Radiother Oncol 1:1-22, 1983, with permission.)



**Figure 22.5.** Highly speculative illustration attempting to extrapolate the experimental data for early- and late-responding tissue in rats and mice to principles that can be applied in clinical radiotherapy. The extra dose required to counter proliferation in early-responding tissues begins to increase after a few weeks into a fractionated regimen, certainly during the time course of conventional therapy. By contrast, conventional protocols are never sufficiently long to include the proliferation of late-responding tissues.

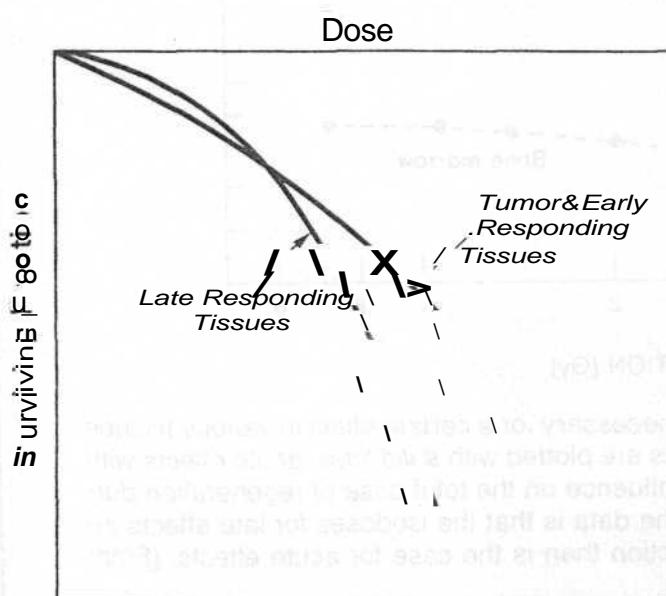
### THE SHAPE OF THE DOSE-RESPONSE RELATIONSHIP FOR EARLY- AND LATE-RESPONDING TISSUES

Clinical and laboratory data suggest that there is a consistent difference between early- and late-responding tissues in their responses to changing fractionation patterns. If fewer and

larger dose fractions are given, late reactions are more severe, even though early reactions are matched by an appropriate adjustment in total dose. This dissociation can be interpreted as differences in repair capacity or shoulder shape of the underlying dose-response curves. The dose-response relationship for late-responding tissues is more curved than that for early-responding tissues. In terms of the linear-quadratic relationship between effect and dose this translates into a larger  $a/p^1$  ratio for *early* than *late* effects. The difference in the shapes of the dose-response relationships is illustrated in Figure 22.6. The  $a/p^1$  ratio is the dose at which cell killing by the linear ( $a$ ) and quadratic ( $p$ ) components are equal.

For early effects  $a/p$  is large; as a consequence,  $a$  dominates at low doses, so that the dose-response curve has a marked initial slope and does not bend until higher doses. The linear and quadratic components of cell killing are not equal until about 10 Gy (1,000 rad). For late effects  $a/p$  is small, so that the  $p$  term has an influence at low doses. The dose-response curve bends at lower doses to appear more curvy; the linear and quadratic components of cell killing are equal by about 2 Gy (200 rad).

Dose-response curves for organ function must be distinguished clearly from those for clonogenic cell survival. The distinction is not



**Figure 22.6.** The dose-response relationship for late-responding tissues is more curved than for early-responding tissues. In the linear-quadratic formulation this translates into a larger  $a/p$  for early than for late effects. The ratio  $a/p$  is the dose at which the linear ( $a$ ) and the quadratic ( $p$ ) components of cell killing are equal: that is,  $aD = pD^2$ . (Based on the concepts of Withers.)

a trivial one. Organ function obviously is related more to the proportion of functional cells remaining in an irradiated organ at a particular time than to the proportion of clonogenic (stem) cells. The dose-effect curves for clonogenic cells tend to be straight, with relatively small shoulders, whereas dose-effect relations for organ function tend to be more curved with a larger shoulder. It is, of course, the dose-response curves for organ function that are more relevant to the tolerance of normal tissues.

There are three pieces of information from clinical experience and animal studies that represent circumstantial evidence for the conclusion that the shape of the dose-response relationship differs for early- and late-respond-

ing tissues. First, if a fractionation scheme is changed in clinical practice from many small doses to a few large fractions and the total dose is titrated to produce equal early effects, the treatment protocol involving a few large fractions results in *more severe late effects*. There is an abundance of clinical evidence for the truth of this statement.

Second, clinical trials of hyperfractionation, in which two doses are delivered per day for 6 or 7 weeks, appear to result in greatly reduced late effects if the total dose is titrated to produce equal or possibly slightly more severe acute effects. Tumor control is the same or slightly improved. This important clinical observation has been made in a number of

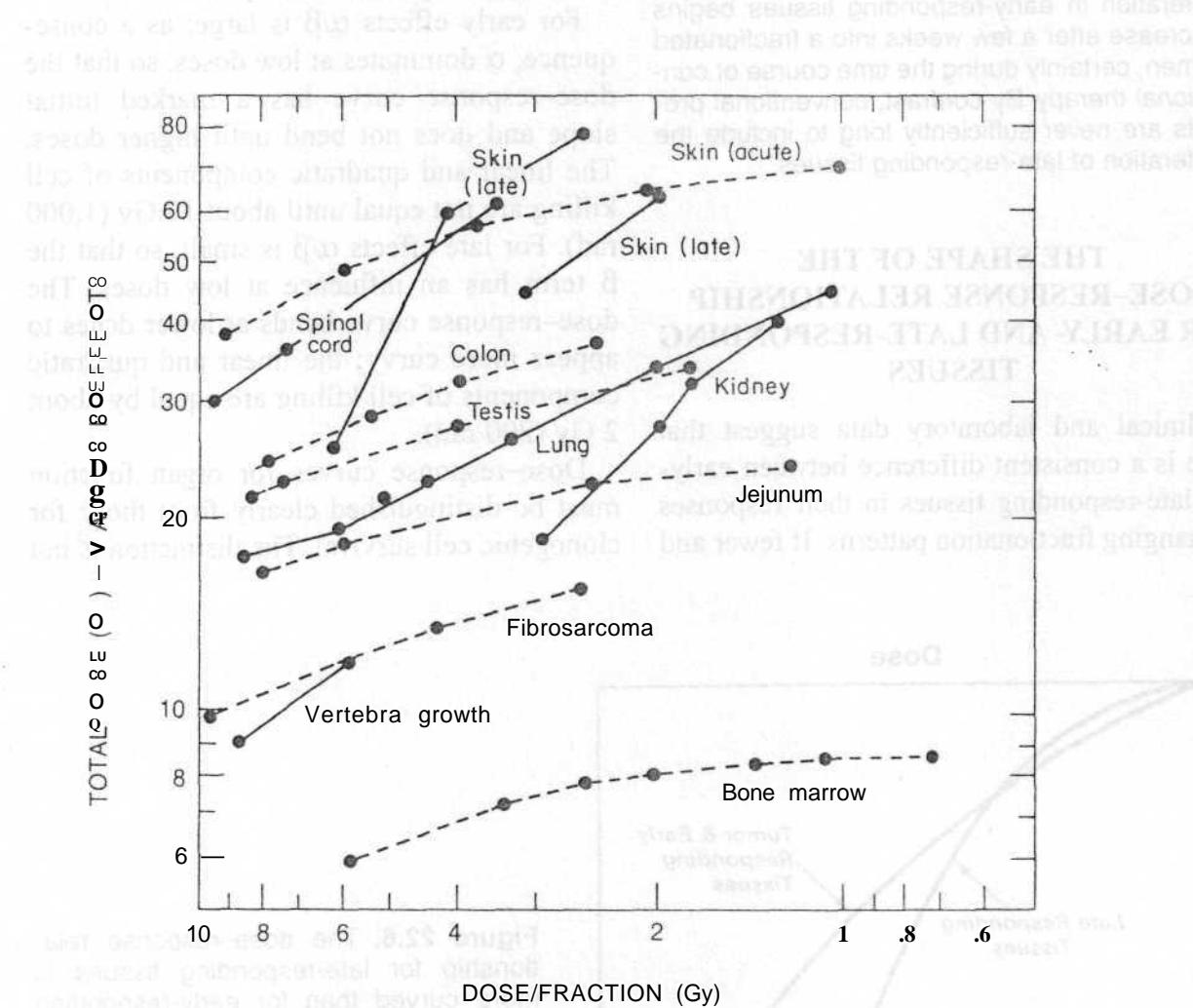


Figure 22.7. Isoeffect curves in which the total dose necessary for a certain effect in various tissues is plotted as a function of dose per fraction. Late effects are plotted with solid lines, acute effects with dashed lines. The data were selected to exclude an influence on the total dose of regeneration during the multifraction experiments. The main point of the data is that the isodoses for late effects increase more rapidly with a decrease in dose per fraction than is the case for acute effects. (From Withers HR: Cancer 55:2086, 1985, with permission.)

centers and again is compatible with the same difference in shape of dose-response curves between early- and late-responding tissues. Late-effect tissues are more sensitive to changes in fractionation patterns than early-responding tissues.

Third, in experiments with laboratory animals the isoeffect curves (*i.e.*, dose *versus* number of fractions to produce an equal biologic effect) are *steeper* for a range of late effects than for a variety of acute effects. The data are shown in Figure 22.7, in which early effects are represented by, for example, skin desquamation or jejunal crypt colonies, and late effects are represented by, for example, the lung or spinal cord injury. Table 22.1 is a summary of the values of  $\alpha/\beta$  for a number of early- and late-responding tissues. The important result is that for early-responding tissues  $\alpha/3$  (*i.e.*, the dose at which single- and multiple-event cell killing are about equal) occurs at the dose of about 10 Gy (1,000 rad). By contrast,  $\alpha/\beta$  for late-responding tissues is about 2 Gy (200 rad). These results come from experiments in small laboratory animals. The values of  $\alpha/3$  shown in Table 22.1 come from experiments in which the reciprocal of the total dose is plotted against the quadratic relationship in biologic systems in which it is possible to observe equal effects from various fractionation regimens, even though single-cell dose-survival curves cannot be generated (Fig. 18.26).

The parameters derived from curves reconstructed from multifraction experiments are

specifically relevant to the endpoint measured in each experiment, whether it is a proportion of clonogenic cells or a stated reduction in organ function. The dose-response curve that is constructed from multifraction experimental data by making simple assumptions is a functional dose-response curve, deduced from data in which repair after each fractional dose is basically the quantity being measured. It is just such functional dose-response curves that are required to elucidate the relationship between tolerance dose in radiotherapy and size of dose per fraction, with overall time considered separately.

### POSSIBLE EXPLANATIONS FOR THE DIFFERENCE IN SHAPE OF DOSE-RESPONSE RELATIONSHIPS FOR EARLY-AND LATE-RESPONDING TISSUES

The radiosensitivity of a population of cells varies with the distribution of cells through the cycle. In general, cells are most resistant in late S phase; slowly growing cells with a long cycle, however, may have a second resistant phase in the early Gi phase, which may be termed Go if the cells are out of cycle. Thus, two quite different cell populations may be radioresistant:

1. A population proliferating so fast that S phase occupies a major portion of the cycle.
2. A population proliferating so slowly that many cells are in early Gi or not proliferating at all, so that many resting cells are in Go.

It is thought that many late-responding normal tissues are resistant, owing to the presence of many resting cells. This type of resistance applies particularly to small doses per fraction and disappears at larger doses per fraction.

If resistance results from the presence of many cells in S phase in a rapidly proliferating population, redistribution occurs through all the phases of the cell cycle, which can be considered a "self-sensitizing" activity. The

**TABLE 22.1.** Ratio of Linear to Quadratic Terms From Multifraction Experiments

Reactions	$\alpha/3$ , Gy
Early	
Skin	9-12
Jejunum	6-10
Colon	10-11
Testis	12-13
Callus	9-10
Late	
Spinal cord	1.7-4.9
Kidney	1.0-2.4
Lung	2.0-6.3
Bladder	3.1-7

fast proliferation itself is a form of resistance, because the new cells produced by division offset those killed by the dose fractions. This applies to *acutely responding tissues* and also to *tumors*. Proliferation occurring during a protracted, fractionated regimen helps to spare normal tissues but, of course, is a potential danger as far as the tumor is concerned. This is discussed subsequently in this chapter.

### FRACTION SIZE AND OVERALL TREATMENT TIME: INFLUENCE ON EARLY- AND LATE-RESPONDING TISSUES

The difference in shape of the dose-response relationship for early- and late-responding tissues leads to an important axiom:

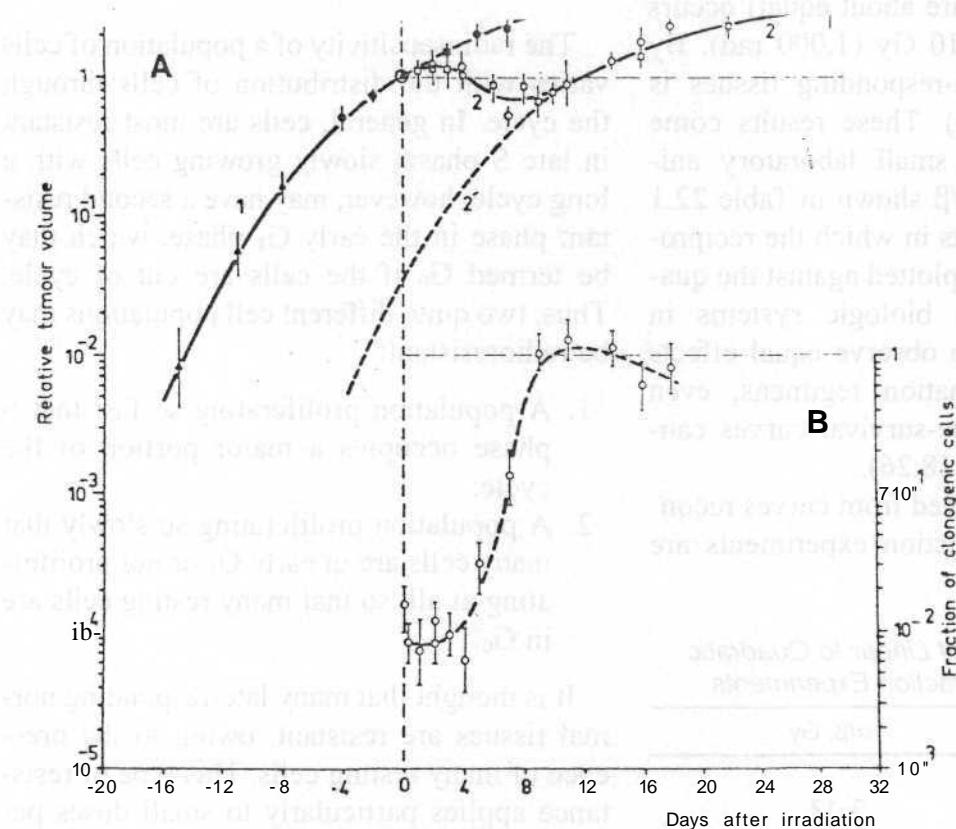
Fraction size is the dominant factor in determining late effects; overall treatment time has little influence. By contrast, fraction size and overall treatment time both determine the response of acutely responding tissues.

It is remarkable that neither clinical radiation oncologists nor experimental radiobiologists came to recognize this simple fact before the mid-1980s.

### ACCELERATED REPOPULATION

Treatment with any cytotoxic agent, including radiation, can trigger surviving cells (clonogens) in a tumor to divide faster than before. This is known as **accelerated repopulation**.

Figure 22.8 illustrates this phenomenon in a transplanted rat tumor. Figure 22.8A shows



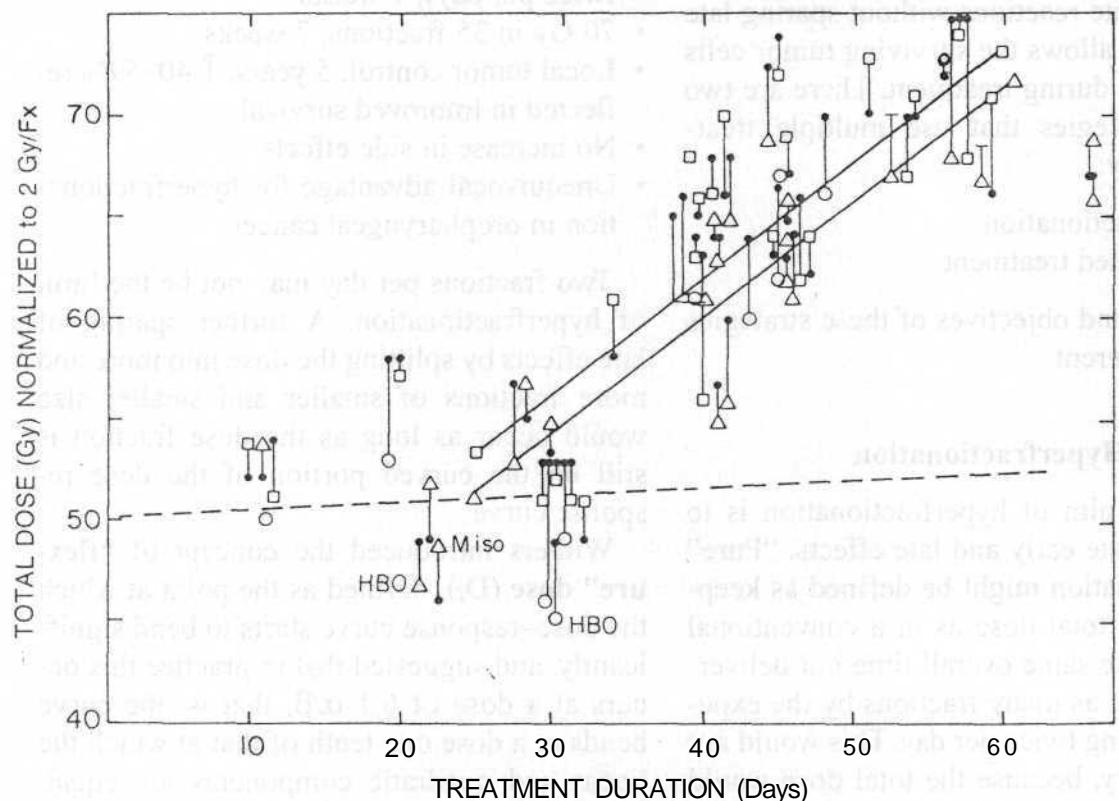
**Figure 22.8.** Accelerated repopulation. Growth curves of a rat rhabdomyosarcoma showing the shrinkage, growth delay, and subsequent recurrence following treatment with a single dose of 20 Gy (2,000 rad) of x-rays. A: Curve 1: Growth curve of unirradiated control tumors. Curve 2: Growth curve of tumors irradiated at time  $t = 0$ , showing tumor shrinkage and recurrence. B: Variation of the fraction of clonogenic cells as a function of time after irradiation, obtained by removing cells from the tumor and assaying for colony formation in vitro. (From Hermens AF, Barendsen GW: Eur J Cancer 5:173-189, 1969, with permission.)

the overall growth curve for this tumor, together with the shrinkage and regrowth that occurs after a single dose of 20 Gy (2,000 rad) of x-rays. Figure 22.8B shows the proliferation of individual surviving cells (*i.e.*, clonogenic cells) that, after treatment, are dividing with a cycle time of 12 hours. The important point to note is that during the time that the tumor is overtly shrinking and regressing, the surviving clonogens are dividing and increasing in number more rapidly than ever.

There is evidence for a similar phenomenon in human tumors. Withers and his colleagues surveyed the literature on radiotherapy for head and neck cancer and estimated the dose to achieve local control in 50% of cases as a function of the overall duration of fractionated treatment. The results are summarized in Figure 22.9. The analysis suggests

that clonogen repopulation in this human cancer accelerates at about 28 days after the initiation of radiotherapy in a fractionated regimen. A dose increment of about 0.6 Gy (60 rad) per day is required to compensate for this repopulation. Such a dose increment is consistent with a 4-day clonogen doubling rate, compared with a median of about 60 days for unperturbed growth.

The conclusion to be drawn from this is that radiotherapy, at least for head and neck cancer, and probably in other instances, also, should be completed as soon after it has begun as is practicable. It may be better to delay initiation of treatment than to introduce delays during treatment. If overall treatment time is too long, the effectiveness of later dose fractions is compromised because the surviving clonogens in the tumor have been triggered into rapid repopulation.



**Figure 22.9.** Doses to achieve local control in 50% of cases ( $TCD_{50}$ ), as a function of overall treatment time, for squamous cell tumors of the head and neck. The data points include many published results from the literature, including high-pressure oxygen trials (HBO), and the trial of misonidazole (Miso). The dashed line shows the rate of increase in  $TCD_{50}$  predicted from a 2-month clonogen doubling rate. (From Withers HR, Taylor JMG, Maciejewski B: The hazard of accelerated tumor clonogen repopulation during radiotherapy. *Acta Oncol* 27:131-146, 1988, with permission.)

The experimental data referred to here all relate to radiotherapy. It might be anticipated, however, that similar considerations would apply to chemotherapy, or to a combination of radiotherapy and chemotherapy. There is evidence in some human malignancies that radiotherapy produces poorer results if preceded by a course of chemotherapy. It may be that accelerated repopulation, triggered by the chemotherapy, is the explanation.

### MULTIPLE FRACTIONS PER DAY

We are now in a position to sum up the pros and cons of fractionation and prolongation of treatment in a much more sophisticated way than would have been possible at the beginning of this chapter. The advantages of prolongation of treatment are to spare early reactions and to allow adequate reoxygenation in tumors. Excessive prolongation, however, has two disadvantages: It can decrease deceptively the acute reactions without sparing late injury, and it allows the surviving tumor cells to proliferate during treatment. There are two separate strategies that use multiple treatments per day:

1. Hyperfractionation
2. Accelerated treatment

The aims and objectives of these strategies are quite different.

#### Hyperfractionation

The basic aim of hyperfractionation is to further separate early and late effects. "Pure" hyperfractionation might be defined as keeping the same total dose as in a conventional regimen in the same overall time but delivering it in twice as many fractions by the expedient of treating twice per day. This would not be satisfactory, because the total dose would need to be increased if the dose per fraction is decreased. In practice, then, "impure" hyperfractionation involves an increase in the total dose and sometimes a longer overall time, as well as many more fractions delivered twice per day. The intent is to further reduce late ef-

fects but achieve the same or better tumor control and the same or slightly increased early effects.

A large controlled clinical trial of hyperfractionation was conducted by the European Cooperative Group (EORTC) in the treatment of head-and-neck cancer. A hyperfractionated schedule of 80.5 Gy delivered in 70 fractions (1.15 Gy twice per day) over a period of 7 weeks was compared with a conventional regimen of 70 Gy delivered in 35 fractions of 2 Gy over 7 weeks. Local tumor control at 5 years was increased from 40 to 59%, and this was reflected in improved survival. There was no increase reported in late effects or complications. It was concluded that hyperfractionation confers an unequivocal advantage in the treatment of oropharyngeal cancer.

The hyperfractionation results of EORTC 22791 are summarized as follows:

- Compare 80.5 Gy in 70 fractions (1.15 Gy twice per day), 7 weeks
- 70 Gy in 35 fractions, 7 weeks
- Local tumor control, 5 years, T 40-59% reflected in improved survival
- No increase in side effects
- Unequivocal advantage for hyperfractionation in oropharyngeal cancer

Two fractions per day may not be the limit of hyperfractionation: A further sparing of late effects by splitting the dose into more and more fractions of smaller and smaller size would occur as long as the dose fraction is still on the curved portion of the dose-response curve.

Withers introduced the concept of "flexure" dose ( $D_f$ ), defined as the point at which the dose-response curve starts to bend significantly, and suggested that in practice this occurs at a dose of 0.1  $\text{oc}/\text{P}$ ; that is, the curve bends at a dose one tenth of that at which the linear and quadratic components are equal. Values of  $a/p$  are 6 to 12 Gy (600-1,200 rad) for early-responding tissues and 1 to 5 Gy (100-500 rad) for late-responding tissues. The flexure dose, therefore, with an  $a/p$  value of 0.1, is remarkably small: 0.6 to 1.2 Gy (60-120 rad) for early-responding tissues and

0.1 to 0.5 Gy (10-50 rad) for late-responding tissues, such as the spinal cord, kidney, lung, or late contraction in skin.

The important conclusion is that to exploit fully the sparing of late damage, doses per fraction as low as this should be used. It should be emphasized that there would not be further sparing of early-reacting tissues or, indeed, of tumors by such low doses per fraction. To not prolong overall treatment time too much, these small doses per fraction would necessitate three or even four fractions per day.

### Accelerated Treatment

The alternative strategy to hyperfractionation is **accelerated treatment**. "Pure" accelerated treatment might be defined as the same total dose delivered in half the overall time by the expedient of giving two or more fractions each day. In practice, it is never possible to achieve this, because the acute effects become limiting. It is necessary either to interpose a rest period in the middle of the treatment or to reduce the dose slightly with acute effects as the limiting factor. The intent of this accelerated treatment strategy is to reduce repopulation **in** rapidly proliferating tumors. There should be little or no change in the late effects, because the number of fractions and the dose per fraction are unaltered.

A large prospective randomized clinical trial of accelerated treatment for head-and-neck cancer, except oropharynx, was carried out by the EORTC. The accelerated treatment consisted of 72 Gy in 45 fractions (three fractions of 1.6 Gy per day) over a total time of 5 weeks, with a rest of two weeks in the middle. The conventional control arm consisted of 35 fractions of 2 Gy, a total dose of 70 Gy in 7 weeks. The results of this trial showed a 15% increase in locoregional control, which did not translate, however, into a survival advantage. As expected, acute effects were increased significantly, but the observed increase **in** late effects was decidedly not expected; some involved complications that proved to be lethal.

This EORTC trial and several others testing accelerated treatment show that attempting to keep the total dose as high as 66 to 72 Gy but shorten the overall time by as much as 2 to 3 weeks from a conventional 6 or 7 weeks leads to serious late complications. There are probably two reasons for this: First, the late effects observed are "consequential" late damage, that is, late damage developing out of the very severe acute effects. Second, there is incomplete repair between dose fractions if several fractions per day are given. This is especially likely for protocols involving three fractions per day, in which any unrepaired damage in the first interval accumulates in the second interval in each day, and also because intervals between fractions of only 4 hours were used in the early years of the EORTC trial.

The EORTC 22851 results regarding accelerated treatment are as follows:

- Head-and-neck cancer except oropharynx
- Cf 72 Gy in 45 fractions (1.6 Gy, three fractions per day), 5 weeks, 70 Gy in 35 fractions, 7 weeks
- 15% increase in locoregional control, no survival advantage
- Increased acute effects (expected)
- Unexpected increase in late effects including lethal complications
- Pure accelerated treatment should be used with extreme caution.

### Continuous Hyperfractionated Accelerated Radiation Therapy

A unique and most interesting study of accelerated treatment was carried out at the Mount Vernon Hospital (U.K.), in association with the Gray Laboratory. This trial is known as Continuous Hyperfractionated Accelerated Radiation Therapy (CHART). The protocol consisted of 36 fractions over 12 consecutive days, with three fractions delivered daily with an interfraction interval of 6 hours. The dose per fraction was 1.4 to 1.5 Gy, to a total dose of 50.4 to 54 Gy. By conventional standards, the total dose was very low, but, of course, it was delivered **in** a very short time. The strategy was based on a low

dose per fraction to minimize late effects, and a very short overall time to minimize tumor proliferation. The results of the CHART protocol showed good local tumor control with severe acute reactions. It was claimed that patients favored the protocol because treatment was concluded quickly. The incidence of late effects in general did not increase and by some measures actually decreased. The notable exception was damage to the spinal cord. Several myelopathies were recorded at total doses of 50 Gy, the probable cause being that an interfraction interval of 6 hours is not sufficient for the full repair of sublethal damage in this tissue.

Characteristics of continuous hyperfractionated accelerated radiation therapy in CHART included:

- Low dose/fractionation: 36 fractions
- Short overall time: 12 consecutive days
- No gap in treatment: three fractions per day at 6-hour intervals
- Three fractions per day: 1.4 to 1.5 Gy per fraction, 50.4 to 54 Gy total

CHART is the only one of the "new" fractionation schedules that results in a *lower* incidence of late complications. CHART'S severe but tolerable acute reactions did not translate into late sequelae, probably because the total dose (50-54 Gy) was so low. Effectiveness in tumor control was not lost even at this low dose because the shortening of overall time was extreme, minimizing tumor-cell proliferation. Compliance was also high with CHART because acute reactions did not peak and become uncomfortable until after the end of treatment. If at least as good results as conventional 6- to 7-week treatments can be obtained with less late morbidity, using a short course of only 12 days, that would seem to be a step forward and a likely reason why patients voiced approval of the idea of getting the treatment completed quickly.

The results of CHART can be summarized as follows:

- Local tumor control is good because overall time is short.

- Acute reactions are brisk but peak after treatment is completed.
- Most late effects are acceptable because the dose per fraction is small.
- An exception is the spinal cord: Several myelopathies occurred at 50 Gy because the time between fractions (6 hours) was too short.

### **Accelerated Hyperfractionated Radiation Therapy while Breathing Carbogen and with the Addition of Nicotinamide**

The last experimental protocol that deserves mention is Accelerated Hyperfractionated Radiation Therapy while Breathing Carbogen and with the Addition of Nicotinamide (ARCON). The strategy was to accelerate treatment to avoid tumor proliferation, hyperfractionate (small doses per fraction) to minimize late effects, and add carbogen breathing to overcome chronic hypoxia and nicotinamide to overcome acute hypoxia. Clinical trials to test this complex but imaginative protocol are underway in Europe. Early results of a trial of ARCON in the Netherlands, involving advanced laryngeal cancer, showed spectacular results compared with historical controls. Results of a prospective randomized trial have yet to be published.

Characteristics of ARCON are summarized as follows:

- Accelerated to overcome proliferation
- Hyperfractionated to spare normal tissues
- Carbogen breathing to overcome chronic hypoxia
- Nicotinamide to overcome acute hypoxia

### **The Time Interval between Multiple Daily Fractions**

One thing the two strategies of hyperfractionation and accelerated treatment have in common is that both involve multiple fractions per day, and in this context it is important to ensure that the fractions are separated by a sufficient time interval for the effects of the doses to be independent: that is, for the re-

pair of sublethal damage from the first dose to be complete before the next dose is delivered. With cells cultured *in vitro*, the half-time of repair is usually about 1 hour, although there is evidence that for cells of human origin cultured *in vitro*, it may vary widely from a few minutes to several hours. For normal tissues *in vivo* it is more difficult to make precise estimates, but it has been inferred from fractionation experiments that the repair of sublethal damage may be very much slower in late-responding tissues. The most pertinent and remarkable evidence comes from twice-a-day trials by the Radiation Therapy Oncology Group that indicate that, for a given total dose delivered in a given number of fractions, the incidence of late effects was worse for interfraction intervals less than 4 hours compared with interfraction intervals longer than 6 hours. These data imply that the repair of sublethal damage in late-responding tissues is slow, and so current wisdom dictates an interfraction interval of 6 hours or more if multiple fractions per day are used. Indeed, the CHART experience clearly indicates that even 6 hours is not sufficient for the spinal cord, a late-responding tissue in which, it appears, sublethal damage repair has a very slow component. This is radiobiology learned from the clinic.

### **T<sub>pot</sub> and Accelerated Treatment**

Early results of a much-publicized study of the EORTC appeared to show the usefulness of measuring cell kinetic parameters as a predictive assay in patients receiving radiotherapy for head-and-neck cancer. In a comparison of accelerated with conventional radiotherapy, each patient was given intravenously a tracer dose of the thymidine analogue iododeoxyuridine.

A tumor biopsy was performed between 4 and 8 hours after administration of the iododeoxyuridine, and a specimen was sent for flow-cytometric analysis to estimate T<sub>pot</sub>, as described in Chapter 23 on cell, tissue, and tumor kinetics. The conventional radiotherapy arm consisted of daily 2-Gy fractions, whereas

the accelerated radiotherapy arm consisted of 1.6-Gy fractions given three times per day with 4 hours between fractions. In the accelerated radiotherapy arm, a gap of 2 weeks followed the first week of treatment to allow for normal tissue recovery, followed by a further 2 weeks of treatment. Patients in the two protocol arms received a similar total dose, but it was given in different overall times: 70 Gy in 7 weeks for the conventional and 72 Gy in 5 weeks for the accelerated treatment.

Patients were divided retrospectively into those with "fast"-growing tumors (*i.e.*, T<sub>pot</sub> < 4 days) or "slow"-growing tumors (*i.e.*, T<sub>pot</sub> > 4 days). For the slow-growing tumors, there was no detectable difference between the results of conventional and accelerated treatment. For fast-growing tumors, however, the accelerated treatment resulted in substantially better local control than the conventional protocol and indeed produced results comparable to those obtained for slow-growing tumors.

Regrettably, the predictive power of T<sub>pot</sub> disappeared when the study matured and a larger number of patients were studied for a longer period of time. A number of leads are being followed, but there is no consensus at the present time of predictive assays, based on kinetic parameters, that can guide in the choice of altered fractionation patterns.

### **LESSONS LEARNED FROM FRACTIONATION STUDIES**

A number of lessons have been learned from the clinical trials that have been performed to test the usefulness of altered fractionation patterns:

*First*, hyperfractionation appears to confer an unequivocal benefit in the treatment of head-and-neck cancer, in terms of both local control and survival, without any increase in late sequelae. By contrast, caution is needed in the application of accelerated treatment, because the EORTC trials showed an unexpected increase in serious late complications. Particular caution is necessary if the spinal cord is in the

treatment field for twice-a-day treatments, because repair of sublethal damage has a slow component in this tissue.

*Second*, late effects depend primarily on total dose and dose per fraction; overall time within the usual therapeutic range has little influence.

*Third*, overall treatment time affects both acute effects and tumor control. Gaps in treatment should be avoided because they lead to an increase in overall time with a concomitant decrease in tumor control.

The importance of overall treatment time is illustrated dramatically by the retrospective analysis by Overgaard of three consecutive trials of the Danish cooperative group. All three trials involved a total dose of 66 to 68 Gy. The first trial was of a split-course regi-

men that extended over a total of 9.5 weeks. The 3-year local control was 32%. The second involved five fractions per week over a treatment time of 6.5 weeks, with a 3-year local control of 52%. The most recent trial included six fractions per week, reducing the overall treatment time to 5.5 weeks and improving the 3-year local control to 62%. There was no change in late effects, but as would be expected, the acute reactions became brisker as the overall time was shortened. The protocols and results of these three trials are illustrated in Figure 22.10. These most interesting data must be viewed with some caution, because the three treatment arms were not in a single randomized study but come from different trials over a period of years, the initial purpose of which was to investigate the usefulness of hypoxic-cell radiosensitizers. They indicate

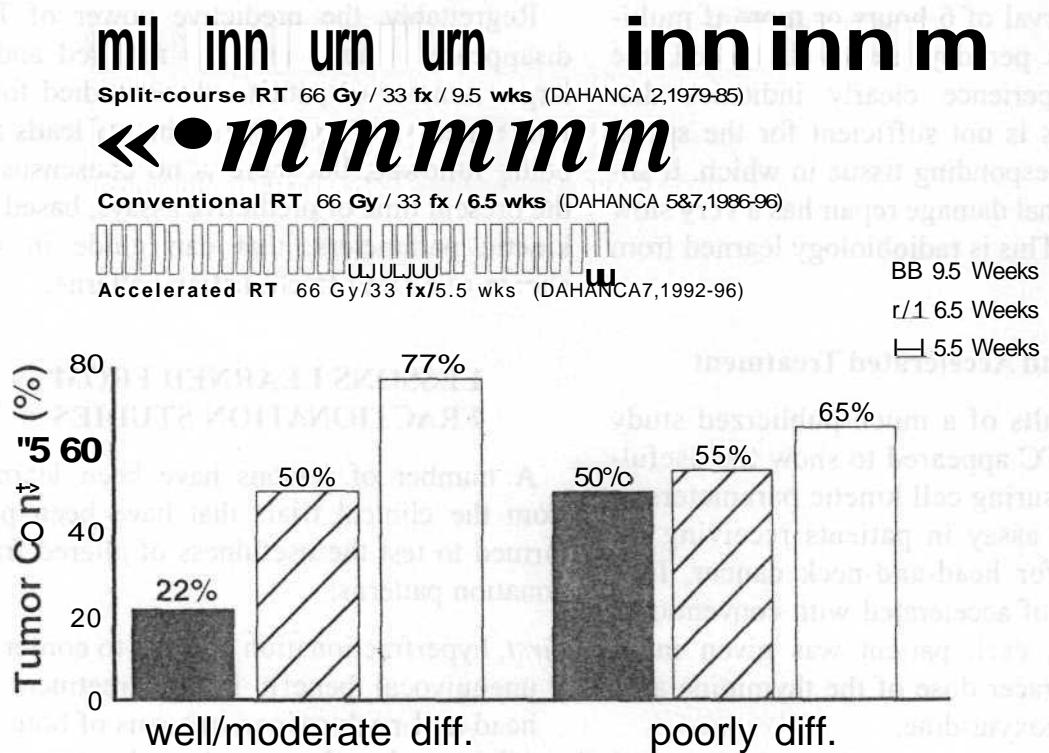


Figure 22.10. Top: Overview of the fractionation schedules used in the three Danish Head and Neck trials. Bottom: Relationship among histopathologic grading, overall treatment time, and local tumor control from the three trials. Only the well and moderately differentiated tumors were significantly influenced by overall treatment time. (Adapted from Overgaard J, Sand Hansen H, Overgaard M, et al.: Importance of overall treatment time for the outcome of radiotherapy in head and neck carcinoma: Experience from the Danish Head and Neck Cancer Study. In Proceedings of ICRO/ÖGRO 6, 6th International Meeting on "Progress in Radio-Oncology," Salzburg, Austria, 13-17 May 1998, pp 743-752. Bologna, Monduzzi Editore S.p.A., 1998, with permission.)

TABLE 22.2. *Importance of Overall Treatment Time*

Total Dose(Gy)	Dose(Gy)	Comment	Overall Time(weeks)	3-Year Local Control
66-68	2	Split course	9 1/2	32
66-68	2	5fr/wk	6 1/2	52%
66-68	2	6fr/wk	5 1/2	62%

Note: DAHANCA trials show improved locoregional control with shorter overall time—no increase in late effects

strongly, nevertheless, that in the case of relatively rapidly growing tumors, such as head-and-neck cancer, overall treatment time can be a dominant factor in determining outcome (Table 22.2).

One of the major lessons to be learned from fractionation studies is that local control is lost if overall treatment time is prolonged. Since it first was proposed independently in the 1980s by Withers and by Fowler, it is now well documented for head-and-neck cancer that local control is reduced by about 0.4 to 2.5% for each day that the overall treatment time is prolonged. This does not differ much from the other way of expressing the same problem, namely that (after the first 4 weeks of a fractionated schedule) the first 0.61 Gy of each day's dose fraction is required to overcome proliferation from the previous day. An equally solid estimate can be made from data for carcinoma of the cervix, in which a mean of 0.5% local control (range 0.3-1.1%) is lost for each day that the overall time is prolonged.

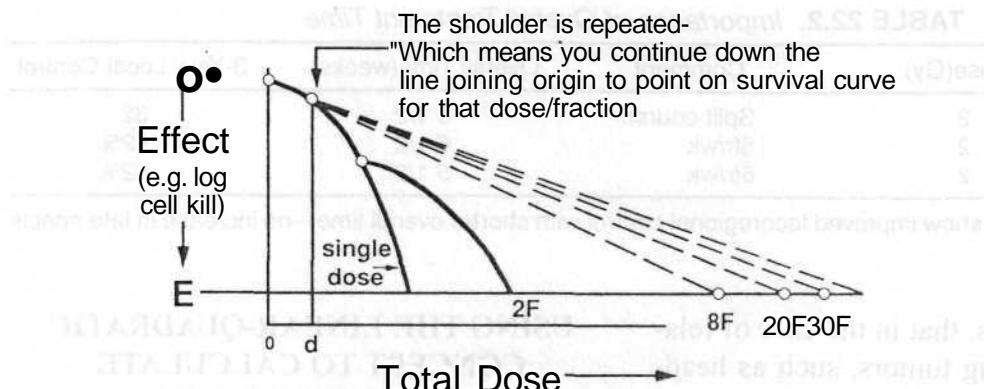
Rapid proliferation does not occur for carcinoma of the breast or prostate, so overall treatment time is not so critical. Although the potential tumor-doubling time ( $T_{pot}$ ) has not proved to be useful as a predictive assay for individual patients (Chapter 23), mean  $T_{pot}$  values for groups of patients are in accord with the importance, or otherwise, of overall treatment time. For example, the  $T_{pot}$  for prostate cancer is about 28 days and for breast cancer about 14 days; in both cases, overall treatment time has not been found to be critical. This can be contrasted with head-and-neck cancer, in which the mean  $T_{pot}$  can be as short as 4 days and, as we have seen, overall treatment time is an important factor governing tumor control.

### USING THE LINEAR-QUADRATIC CONCEPT TO CALCULATE EFFECTIVE DOSES IN RADIOTHERAPY

It is often useful in practice to have a simple way to compare different fractionation regimens and to assign them a numeric score. For many years the NSD system developed by Ellis and colleagues was used widely. It proved useful for assessing modest changes in fractionation but fell into dispute when extrapolated beyond the data range on which it was based. The linear-quadratic model is now more widely used and has received greater acceptance. This section was suggested by Dr. Jack Fowler; the format is based on tutorials that he has given at the American Society of Therapeutic Radiology and Oncology and at the European Society of Therapeutic Radiology and Oncology.

Use of the linear-quadratic model, with appropriate values for the parameters,  $a$  and  $(3)$ , emphasizes the difference between early- and late-responding tissues and the fact that it is never possible to match two different fractionation regimens to be equivalent for both. Calculations of this sort, although a useful guide for residents in training or for research purposes, are not to be considered a substitute for clinical judgment and experience. They are presented only as examples.

Figure 22.11 illustrates the familiar way in which biologic effect as a function of dose varies with the number of fractions into which the radiation is delivered—always assuming that the fractions are spaced sufficiently to allow full repair of sublethal radiation damage. For a multifraction regimen, the shoulder of the curve has to be repeated many times, and as a consequence the effective dose-response



**Figure 22.11.** Graph illustrating that, if the dose-response relationship is linear-quadratic in form for graded single doses, the effective dose-response curve for a multifraction regimen approaches an exponential function of dose for many doses. The effective dose-response relationship is a straight line from the origin through the point on the single dose survival curve corresponding to the daily dose fraction (typically 2 Gy). (Based on the concepts of Fowler.)

relationship is a straight line from the origin through the point on the single-dose survival curve for that dose fraction (typically 2 Gy). This is discussed in Chapter 3. For the linear portion of the curve,  $a$  represents the log<sub>e</sub> of the cells killed per gray. As the curve bends, the quadratic component of cell killing is represented by  $(\beta/3)$ , which is the log<sub>e</sub> of the cells killed per gray squared. This is illustrated in Figure 22.12. The ratio  $a/\beta$  has the dimensions of dose and is the dose at which the linear and quadratic components of cell killing are equal.

For a single acute dose  $D$ , the biologic effect is given by

$$E = \alpha D + \beta D^2 \quad (1)$$

For  $n$  well separated fractions of dose  $d$ , the biologic effect is given by

$$E = n(ad + (\beta/3)d^2) \quad (2)$$

As suggested some years ago by Barendsen, this equation may be rewritten as

$$\begin{aligned} E &= (nd)(\alpha + (\beta/3)d) \\ &= (a)(nd) \left( 1 + \frac{d}{\alpha/\beta} \right) \end{aligned} \quad (3)$$

but  $nd$  equals  $D$ , the total dose, so

$$E = a(D) \text{ (relative effectiveness)}$$

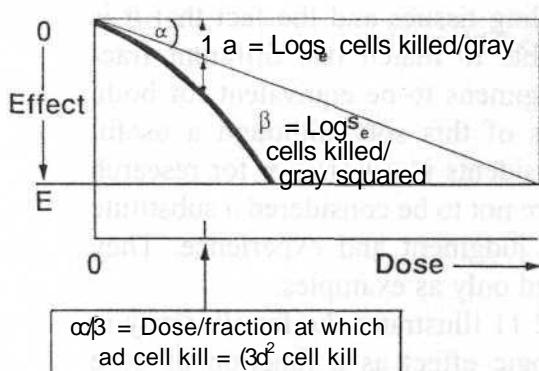
in which the quantity  $1 + [d/(\alpha/\beta)]$  is called *relative effectiveness*. If this equation is divided through by  $a$ , we have

$$\begin{aligned} &= (total \ dose) \times (relative \ effectiveness) \\ &= nd \times \left( 1 + \frac{d}{\alpha/\beta} \right) \end{aligned} \quad (4)$$

The quantity  $E/\alpha$  is the **biologically effective dose** and is the quantity by which different fractionation regimens are intercompared. In words, the final equation is

$$\text{Biologically effective dose} = \frac{(total \ dose)}{(relative \ effectiveness)} \times (relative \ effectiveness) \quad (5)$$

$$\frac{E}{\alpha} = nd \times \left( 1 + \frac{d}{\alpha/\beta} \right) \quad (5)$$



**Figure 22.12.** Graph illustrating the linear-quadratic nature of the radiation cell survival curve,  $S = e^{(\alpha D + \beta D^2)}$  in which  $S$  is the fraction of cells surviving a dose  $D$ ,  $\alpha$  is the number of logs of cell kill per gray from the linear portion of the curve, and  $\beta$  is the number of logs of cell kill per (gray)<sup>2</sup> from the quadratic component. The linear and quadratic components of cell kill are equal at a dose  $D = a/\beta$ .

### Choice of a/p

For calculating the examples that follow, a/p<sup>1</sup> is assumed to be 3 Gy for late-responding tissues and 10 Gy for early-responding tissues. The reader, of course, may substitute other values if they seem to be more appropriate. It should be noted that parts of schedules can be added, that is, (partial effect)<sup>i</sup> and (partial effect)<sup>h</sup>, as in the concomitant boost. It also should be noted that, although it is permissible to compare biologically effective doses for late effects (in grays cubed) of one schedule with another and permissible to compare biologically effective doses for early effects (in Gy<sub>10</sub>) of one schedule with another, it is clearly not permissible or meaningful to compare early with late effects.

### Model Calculations

1. Conventional treatment. 30 fractions of 2 Gy given one fraction per day, 5 days per week, for an overall treatment time of 6 weeks (this is written as 30F x 2 Gy/6 weeks)

$$\begin{aligned} \text{Early effects: } \frac{E}{a} &= (\text{nd}) \left( 1 + \frac{d}{a/p} \right) \\ &= 60 \left( 1 + \frac{2}{10} \right) \\ &= 72 \text{ Gy}_{10} \end{aligned}$$

$$\begin{aligned} \text{Late effects: } \frac{E}{a} &= 60 \left( 1 + \frac{2}{3} \right) \\ &= 100 \text{ Gy}_3 \end{aligned}$$

*Comment:* The subscripts to the biologically effective dose are a reminder that this figure is not in grays and a reminder of the particular values of a/p used in the calculation.

2. Hyperfractionation. 70 fractions of 1.15 Gy given twice daily, 6 hours apart, 5 days per week, for an overall treatment time of 7 weeks, that is, 70F x 1.15 twice daily/7 weeks

$$\begin{aligned} \text{Early effects: } \frac{E}{a} &= (\text{nd}) \left( 1 + \frac{d}{a/\beta} \right) \\ &= 80.5 \left( 1 + \frac{1.15}{10} \right) \\ &= 89.8 \text{ Gy}_{10} \end{aligned}$$

$$\begin{aligned} \text{Late effects: } \frac{E}{a} &= 80.5 \left( 1 + \frac{1.15}{3} \right) \\ &= 111.4 \text{ Gy}_3 \end{aligned}$$

*Comment:* This treatment is much "hotter," that is, more effective, than the conventional for both early and late effects.

3. A one-fraction-a-day control schedule frequently used to compare with hyperfractionation, 35 fractions of 2 Gy given once a day for 5 days a week, for an overall treatment time of 7 weeks, that is, 35F x 2 Gy/7 weeks

$$\begin{aligned} \text{Early effects: } \frac{E}{a} &= (\text{nd}) \left( 1 + \frac{d}{a/p} \right) \\ &= 70 \left( 1 + \frac{2}{10} \right) \\ &= 84 \text{ Gy}_{10} \end{aligned}$$

$$\begin{aligned} \text{Late effects: } \frac{E}{a} &= 70 \left( 1 + \frac{2}{3} \right) \\ &= 116.7 \text{ Gy}_3 \end{aligned}$$

*Comment:* This "control" schedule is not comparable to the hyperfractionation because it is less effective by 7% for early effects, which includes tumor control (84 versus 89.8 Gy<sup>10</sup>) but hotter for late effects by 5% (116.7 versus 111.4 Gy<sup>3</sup>).

4. *Concomitant boost:* 30 fractions of 1.8 Gy given once a day, 5 days a week, and at the same time (concomitant) a boost to a smaller field of 12 fractions of 1.5 Gy once a day; overall treatment time 6 weeks, that is, ([30F x 1.8 Gy] + [12F x 1.5 Gy])/6 weeks (this protocol is much favored at the University of Texas M. D. Anderson Hospital and Tumor Institute; by giving the boost concomitantly, a prolongation of overall time is avoided)

Early effects:

$$\begin{aligned} \frac{E}{a} &= (\text{nd}) \left( 1 + \frac{d}{a/p} \right) \\ &= 54 \left( 1 + \frac{1.8}{10} \right) + 18 \left( 1 + \frac{1.5}{10} \right) \\ &= 84.4 \text{ Gy}_{10} \end{aligned}$$

Late effects:

$$\frac{E}{a} = 54 \left( 1 + \frac{1.8}{3} \right) + 18 \left( 1 + \frac{L_5}{3} \right)$$

$$= 113.4 \text{ Gy}_3$$

*Comment:* The Gy<sub>io</sub> and Gy<sup>^</sup> values should be compared with the comparable figures for the previous schedules given. The concomitant boost is hotter than the conventional schedule for both early and late effects. Compared with hyperfractionation, however, this concomitant boost is almost the same for late effects but less effective for early effects, including tumor control.

5. CHART of 36 fractions of 1.5 Gy given three fractions a day, 6 hours apart, for 12 consecutive days with overall treatment time, 12 days, that is, 36F x 1.5 Gy (3F/day)/12 days

Early effects including tumor:

$$\frac{E}{a} = (nd) \left( 1 + \frac{d}{a/p} \right)$$

$$= 54 \left( 1 + \frac{1.5}{10} \right)$$

$$= 62.1 \text{ Gy}_{10}$$

Late effects:

$$\frac{E}{a} = 54 \left( 1 + \frac{1.5}{3} \right)$$

$$= 81.0 \text{ Gy}_3$$

*Comment:* Direct comparison of CHART with the previous examples in terms of Gy<sub>io</sub> and Gy<sup>^</sup>, is meaningless, because CHART has an overall time of only 12 days compared with 6 or 7 weeks for the other schedules.

### Allowance for Tumor Proliferation

The correction proposed here for tumor proliferation is a crude approximation and should not be taken too seriously. It assumes, among other things, that the rate of cellular proliferation remains constant throughout the overall treatment time.

The number of clonogens (N) at time t is related to the initial number of clonogens (No) by the expression

$$N = No e^{-At} \quad (6)$$

in which A- is a constant related to the potential doubling time of the tumor, T<sub>pot</sub>, by the expression

$$A = \frac{\log_2^2}{T_{\text{pot}}} = 0.693 \quad (7)$$

The decrease in the number of clonogens, because of cell killing by the fractionated radiation regimen, is balanced to some extent by cell division of the surviving clonogens. The biologic effect in equation 2 now becomes

$$E = n(ad + pd^2) - 0.693 \frac{t}{T_{\text{pot}}} \quad (8)$$

The biologically effective dose E/oc becomes

$$\frac{E}{a} = (nd) \left( 1 + \frac{d}{a/\beta} \right) - 0.693 \frac{t}{T_{\text{pot}}} \quad (9)$$

or, in words,

Biologically effective dose

= (total dose) x (relative effectiveness)

$$- - |2fc \cdot r (no. of cell doublings) \quad (10)$$

It is now necessary to assume a value for a, the initial slope of the cell-survival curve, as well as for T<sub>pot</sub>, the potential doubling time of the tumor. A reasonable value for a is 0.3 ± 0.1/Gy. T<sub>pot</sub> may have a value of 2 to 25 days, with a median value of about 5 days.

For typical 6-week (39-day) schedules referred to earlier, proliferation may reduce the biologically effective dose by

$$\frac{E}{a} = \frac{0.693}{0.3} \times \frac{39}{5} = 18^0 \text{ Gy}_{10}$$

Note that because we are concerned with tumor proliferation, the reduction in biologically effective dose is in Gy<sup>10</sup>; that is, an early-effect a/p value is used. By the same token, proliferation during a 7-week protocol (i.e., 46 days) would decrease the biologically effective dose by

$$\frac{E}{a} = \frac{0.693}{0.3} \times \frac{46}{5} = 21.3 \text{ Gy}_{10}$$

CHART calls for three fractions per day over 12 days, and thus proliferation during this time would reduce the biologically effective dose by

$$\frac{E}{a} = \frac{0.693}{0.3} \times \frac{12}{5} = 5.6 \text{ Gy}_m$$

**TABLE 22.3.** Effect of Tumor Proliferation on Biologically Effective Doses Characteristic of Various Treatment Regimens

Protocol	E <sub>0c</sub> Early, i.e., Tumor Gy <sub>0</sub>	Proliferation Correction, Gy <sub>10</sub>	Corrected for Time, Gy <sub>10</sub>
Conventional protocol: 30F x 2 Gy/6 wk (39 d)	72	-18.0	54
Hyperfractionation: 70F x 1.15 Gy/7 wk	89.8	-21.3	68.5
Concomitant boost: (30F x 1.8 Gy) + (12Fx 1.5 Gy)/6 wk (39 d)	84.4	-18.0	66.4
Chart: 36F x 1.5Gy/12d	62.1	-5.6	56.5

Table 22.3 summarizes the effect of tumor proliferation on the biologically effective doses characteristic of the various treatment regimens discussed earlier.

Based on the assumptions made, hyperfractionation results in the largest biologically effective dose and therefore may be expected to result in the best tumor control, followed closely by the concomitant boost schedule. CHART is a much less effective schedule based on a  $T_{pot}$  of 5 days. It is necessary to assume a very fast-growing tumor, with a  $T_{pot}$  of

3 days or less, for CHART to become the most effective schedule.

It must be emphasized again that calculations of this sort should be used only as a guide for residents in training, because they do not in any way replace clinical judgment. It is useful, however, to have a yardstick by which new fractionation schemes may be judged. A number of software packages are being developed to perform on a personal computer calculations of the type described earlier.

### SUMMARY OF PERTINENT CONCLUSIONS

- The four Rs of radiobiology are:
  - Repair of sublethal damage
  - Reassortment of cells within the cell cycle
  - Repopulation
  - Reoxygenation
- The basis of conventional fractionation may be explained as follows. Dividing a dose into a number of fractions *spares* normal tissues because of the repair of sublethal damage between dose fractions and cellular repopulation. At the same time, fractionation *increases* tumor damage because of reoxygenation and reassortment.
- The Strandquist plot is the relation between total dose and overall treatment time. In this context "time" includes the number of fractions. On a double log plot the slope of the line for normal tissues is often close to 0.33.
- The Ellis NSD system made the important contribution of separating the effects of a number of fractions and overall time. The time correction was a power function ( $T^{0.33}$ ) that is far from accurate.
- The extra dose required to counteract proliferation in a normal tissue irradiated in a fractionated regimen is a sigmoidal function of time. No extra dose is required until some weeks into a fractionated schedule.
- The delay before an extra dose is required to counteract the effects of proliferation is much longer for late-responding tissues and is beyond the overall time for conventional radiotherapy schedules.
- Prolonging overall time within the normal radiotherapy range has little sparing effect on *late reactions* but a large sparing effect on *early reactions*.

- The dose-response relationship for late effects is more curvy than for early effects. The ratio  $a/p$  is about 10 Gy (1,000 rad) for early effects and about 2 Gy (200 rad) for late-responding tissues. Consequently, late-responding tissues are more sensitive to changes in fractionation pattern.
- Fraction size is the dominant factor in determining *late effects*; overall treatment time has little influence. By contrast, fraction size and overall treatment time both determine the response of *acutely responding* tissues.
- *Accelerated repopulation* refers to the triggering of surviving cells (clonogens) to divide more rapidly as a tumor shrinks after irradiation or treatment with any cytotoxic agent.
- Accelerated repopulation starts in head-and-neck cancer in humans about 4 weeks after initiation of fractionated radiotherapy. About 0.6 Gy (60 rad) per day is needed to compensate for this repopulation.
- This phenomenon mandates that treatment should be completed as soon as practical once it has started; it may be better to delay the start than to introduce interruptions during treatment.
- The basic aim of hyperfractionation is to further separate early and late effects. The overall treatment time remains conventional at 6 to 8 weeks, but because two fractions per day are used, the total number of fractions is 60 to 80. The dose must be increased, because the dose per fraction is decreased. Early reactions may be increased slightly, tumor control improved, and late effects greatly reduced.
- In accelerated treatment, to reduce repopulation in rapidly proliferating tumors, conventional doses and number of fractions are used; but because two doses per day are given, the overall treatment time is halved. In practice the dose must be reduced or a rest interval allowed because acute effects become limiting.
- Hyperfractionation has been shown in randomized clinical trials of head and neck cancer to improve local tumor control and survival with no increase in acute or late effects.
- Accelerated treatment, the EORTC trial of 72 Gy in 45 fractions (three fractions per day) over 5 weeks showed an increase in local tumor control, but no increase in survival. There was an unexpected increase in late effects, some of which were lethal. The late effects were probably "consequential" late effects, developing out of the severe acute effects. Incomplete repair between fractions also may have been a problem, because the time interval between fractions was too short.
- *CHART* stands for Continuous Hyperfractionated Accelerated Radiotherapy. The protocol consists of 36 fractions over 12 days (three fractions per day) to a total dose of 50.4 to 54 Gy. Tumor control was maintained because of the extreme acceleration of treatment time; late effects were not increased and even may have decreased because of the low dose; and acute effects were severe, but their peak occurred after completion of treatment, so patient compliance was not prejudiced.
- *ARCON* involves accelerated treatment to overcome tumor cell proliferation, hyperfractionation to spare late-responding normal tissues, carbogen breathing to overcome chronic hypoxia<sup>1</sup>, and nicotinamide to overcome acute hypoxia.
- Overall treatment time is a very important factor for fast-growing tumors. In head-and-neck cancer, local tumor control is decreased by 0.4 to 2.5% for each day that the overall treatment time is prolonged. The corresponding figure for carcinoma of the cervix is about 0.5% per day. Such rapid proliferation is not seen in breast or prostate cancer.

- The linear-quadratic concept may be used to calculate the biological effectiveness of various radiotherapy protocols. The useful formula is

$$\text{(Biologic effective dose)} = (\text{total dose}) \times (\text{relative effectiveness})$$

$$E_{\text{ot}} = n \times d \times (1 + \frac{\lambda}{a/p})$$

- An approximate allowance can be made for tumor proliferation during an extended radiotherapy course by assuming a value for  $T_{\text{pot}}$ .

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# 23

## Predictive Assays

### RADIOSENSITIVITY OF NORMAL TISSUES PREDICTIVE ASSAYS FOR TUMORS

### CONCLUSION SUMMARY OF PERTINENT CONCLUSIONS

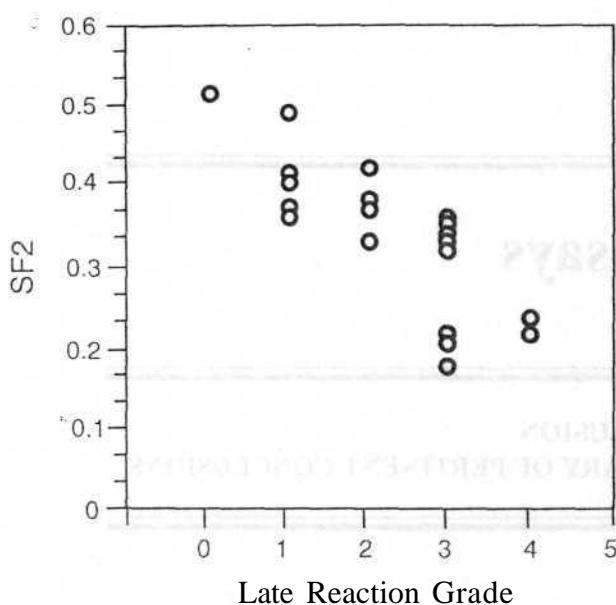
In the routine day-to-day practice of radiotherapy, treatment schedules are designed for the "average" patient with a given type of malignancy at a given site. Although much "lip service" is rendered to the subject, in practice little is done to tailor a treatment schedule to the individual case. The eventual goal of predictive assays is to choose a treatment protocol that is optimal for each individual patient, and that might give a better chance of cure than the conventional therapy. In particular, such assays may one day be used to select patients suitable for new experimental protocols. This chapter is included only after much thought, because, although a wide variety of assays are being explored, none is fully developed or universally accepted. The potential, however, is so great that the resident in training should be aware of the basic ideas.

#### RADIOSENSITIVITY OF NORMAL TISSUES

Among any groups of patients given the same radiation treatment, some show more severe normal-tissue reactions than others, and a small proportion suffer unacceptable late sequelae. There is a powerful incentive to find ways and means to prospectively identify radiosensitive individuals, both to minimize patient suffering and to avoid potential lawsuits.

A number of research groups have addressed the feasibility of predicting normal-tissue tolerance from laboratory measurements of the radiosensitivity of cells taken from biopsies or blood samples. Figure 23.1 shows results of one of the most convincing studies. Fibroblasts were grown out of skin biopsies and the fraction of cells surviving a dose of 2 Gy (SF<sub>2</sub>) determined by a colony assay. The results show a significant correlation with *late* radiation damage to the skin (Fig. 23.1), though, interestingly enough, *not* with acute reactions. The measurements of fibroblast cell survival takes many weeks and is therefore too slow to be used as a predictive assay. Other efforts to identify rapid and reliable alternatives to fibroblast colony formation, such as chromosome damage, micronuclei formation, the comet assay for DNA damage, and so forth, have so far failed to get to first base.

A more likely possibility in the future would be to screen potential radiation therapy patients for mutations in genes that may be involved in radiosensitivity or radioresistance. An obvious first possibility would be that ataxia telangiectasia(AT) heterozygotes are at risk of late effects following radiation therapy, because it is known that AT homozygotes are extremely radiosensitive and cells from AT heterozygotes are slightly more radiosensitive than normal. The problem with this approach



**Figure 23.1.** Data for 20 patients showing the correlation between late reaction to radiation therapy and the *in vitro* radiosensitivity of fibroblasts obtained from a biopsy. SF<sub>2</sub> is the fraction of cells surviving an acute dose of 2 Gy. The correlation is significant with a probability of 0.0001. (Redrawn from the data of Geard et al; Int J Radiat Oncol Biol Phys 27:1173-1179, 1993, with permission.)

is the apparent complexity of the situation. Multiple genes have been identified that influence radiosensitivity in experimental systems, but their importance in patients is unknown. It remains an attractive speculation that, someday in the future, rapid and inexpensive tests will be available to prospectively identify patients who are radiosensitive and likely to suffer unacceptable late effects if given a standard course of radiation therapy.

#### PREDICTIVE ASSAYS FOR TUMORS

Assays to predict the radiation characteristics of a tumor fall into three categories:

1. Intrinsic cellular radiosensitivity
2. Oxygen status
3. Proliferative potential

##### Intrinsic Cellular Radiosensitivity

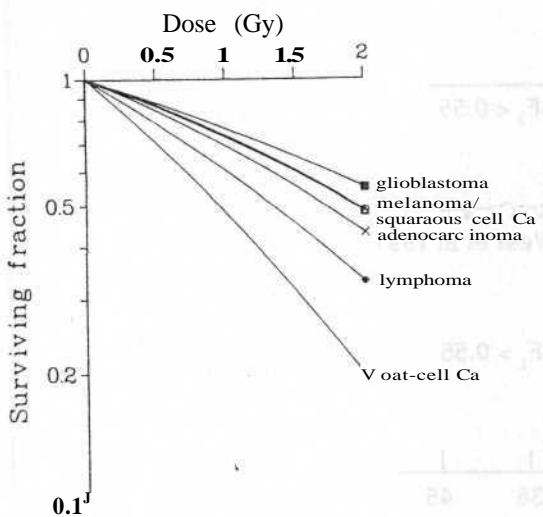
Clonogenic cell survival, assessed by the single-cell plating techniques described in

Chapter 3, long has been considered to be the gold standard for judging the cellular response to anticancer agents, including radiation. The formation of a microscopic colony from a single cell requires sustained cell division and is the ultimate proof of reproductive integrity. Cells taken directly from human tumor biopsy specimens, however, do not grow readily and usually are characterized by a poor plating efficiency (often about 1%). It is not easy to obtain a repeatable estimate of the cell surviving fraction to a dose of, say, 2 Gy.

The possibility of a predictive assay for intrinsic cellular radiosensitivity derives from the many attempts that have been made to correlate the *in vitro* radiosensitivity of *cell lines* derived from human tumors with the clinical responsiveness of tumors of the same histologic group. The most extensive studies are those of Deacon, Peckham, and Steel in the United Kingdom and of Malaise and Fertil in France.

The conclusion from these studies is that the steepness of the initial slope of the survival curve correlates with clinical responsiveness rather than the final slope. This initial region of the survival curve is best characterized by SF<sub>2</sub>. The characteristics of the survival curve at higher doses, designated by the final D<sub>0</sub>, or (3 in the linear-quadratic formula, did not correlate with clinical outcome. Malaise and his colleagues divided tumors into six histologic groups: From the most radioresistant to the most radiosensitive, these are glioblastomas, melanomas, squamous cell carcinomas, adenocarcinomas, lymphomas, and oat cell carcinomas. The order of the SF<sub>2</sub>s correlates with clinical responsiveness. This is illustrated in Figure 23.2. But most important, Malaise and his colleagues demonstrated widely diverse sensitivities within each histologic group, such that the most sensitive glioblastoma had a radiosensitivity similar to the most resistant lymphoma.

These findings are of significant radiobiologic interest because they contradict the widely held view that clinical responsiveness is not related to inherent cellular radiosensitivity.



**Figure 23.2.** Initial portion of the mean representative survival curves for cells from each of six histologic groups of human tumors, showing also the surviving fraction at 2 Gy (SF2). (Data from Malaise EP, Fertil B, Chavaudra N, Guichard M: Distribution of radiation sensitivities for human tumor cells of specific histological types: Comparison of *in vitro* to *in vivo* data. Int J Radiat Oncol 12:617-624, 1986.)

To some extent it also weakens the equally widely held view that differences in responsiveness between tumors must be attributable to differences in hypoxic fraction. It is, however, not of much use to the clinical radiotherapist, who does not need *in vitro* measurements to tell him or her that lymphomas are more radiosensitive than glioblastomas. What the radiotherapist needs is information on the individual patient. These data from Malaise and his colleagues indicate that, because radioresponsiveness may vary with the DNA characteristics of the tumor cells involved, at least between histologic types, then the possibility exists that individual responsiveness may be predicted if a suitable assay were available. The radiobiologic experiments with cell lines derived from human tumors laid the groundwork for predictive assays of radiosensitivity of cells from individual patients.

#### *Growth in Soft Agar: The Courtenay Assay*

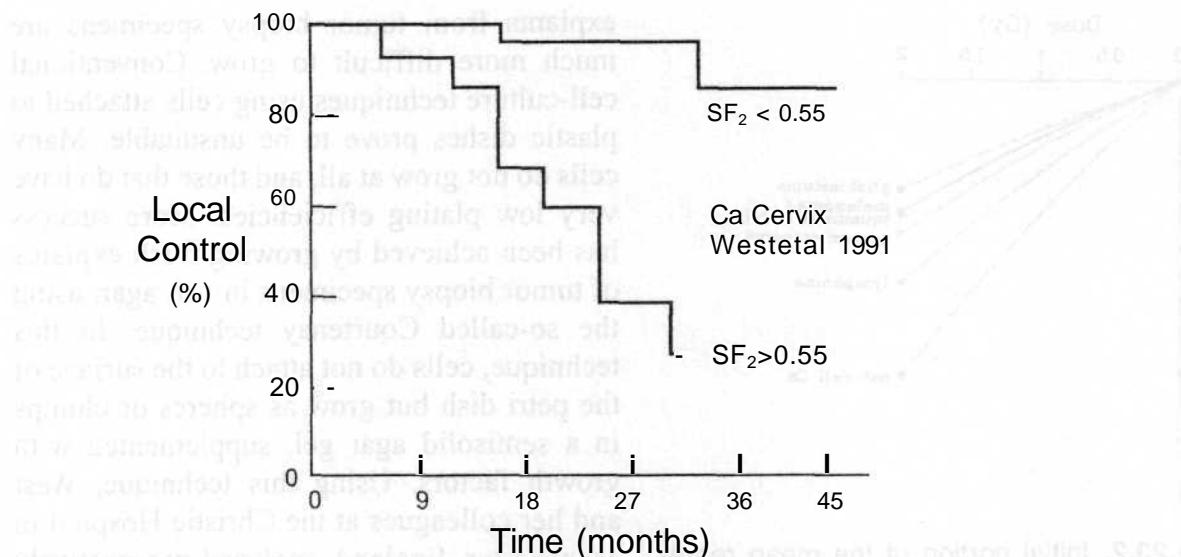
Although cell lines derived from human tumors can be studied using standard *in vitro* plating assays (described in Chapter 3), fresh

explants from tumor biopsy specimens are much more difficult to grow. Conventional cell-culture techniques using cells attached to plastic dishes prove to be unsuitable. Many cells do not grow at all, and those that do have very low plating efficiencies. More success has been achieved by growing fresh explants of tumor biopsy specimens in soft agar, using the so-called Courtenay technique. In this technique, cells do not attach to the surface of the petri dish but grow as spheres or clumps in a semisolid agar gel, supplemented with growth factors. Using this technique, West and her colleagues at the Christie Hospital in Manchester, England, analyzed prospectively for intrinsic radiosensitivity over 50 patients with stage I, II, and III carcinoma of the cervix who were to receive radical radiotherapy. They found that patients with SF2S of greater than 0.55 had significantly lower probabilities of local control than those with SF2S smaller than 0.55. Their data are shown in Figure 23.3. Stage alone was a poorer prognostic factor for local control than radiosensitivity. These findings suggest that intrinsic radiosensitivity may be a useful predictor of local recurrence after radical radiotherapy, but the fact that the best data date from 1991 indicates that there are problems involved.

Because of the difficulties of performing clonogenic assays on fresh explants of tumor cells, several attempts have been made to develop nonclonogenic assays that depend on growth, are easier to handle, and yield usable results more quickly. Growth rate can be assessed quantitatively by several methods, including exclusion of vital dyes and the incorporation of labeled biochemical precursors. Only two are described here.

#### *Colorimetric Assays*

Cells from either an established cell line or a tumor biopsy specimen are plated into 96-well plates, treated with graded doses of radiation or of a cytotoxic drug, and allowed to grow for several days. The cells then are stained in one way or another, with quantitation of cell growth based on the assumption



**Figure 23.3.** Local control curves for carcinoma of the cervix (stages I, II, and III) treated by radiotherapy alone. Patients were separated into two groups according to the intrinsic radiosensitivity of the tumor biopsy, measured *in vitro* by the "Courtenay" assay. The two groups showed surviving fractions at 2 Gy (SF<sub>2</sub>) above and below 0.55. (Adapted from West CML, Davidson SE, Hendry JH, Hunter RD: Prediction of cervical carcinoma response to radiotherapy. Lancet 338:818, 1991, with permission.)

that only *viable* tumor cells are stained. The density of the stain in a given well measured by a spectrophotometer correlates with the number of surviving cells. The technique is much simpler and faster than a clonogenic assay and is amenable to automation.

One of the earliest colorimetric assays was the tetrazolium (MTT) assay. Viable tumor cells reduce a tetrazolium-based compound to a blue formazan product that can be assessed with a spectrophotometer. A stained dish from a typical experiment with the MTT assay is shown in Figure 23.4, together with a typical survival curve. This assay was adopted by the National Cancer Institute to screen large numbers of potential anticancer drugs. It is also useful to identify compounds that are radiosensitizers or radioprotectors.

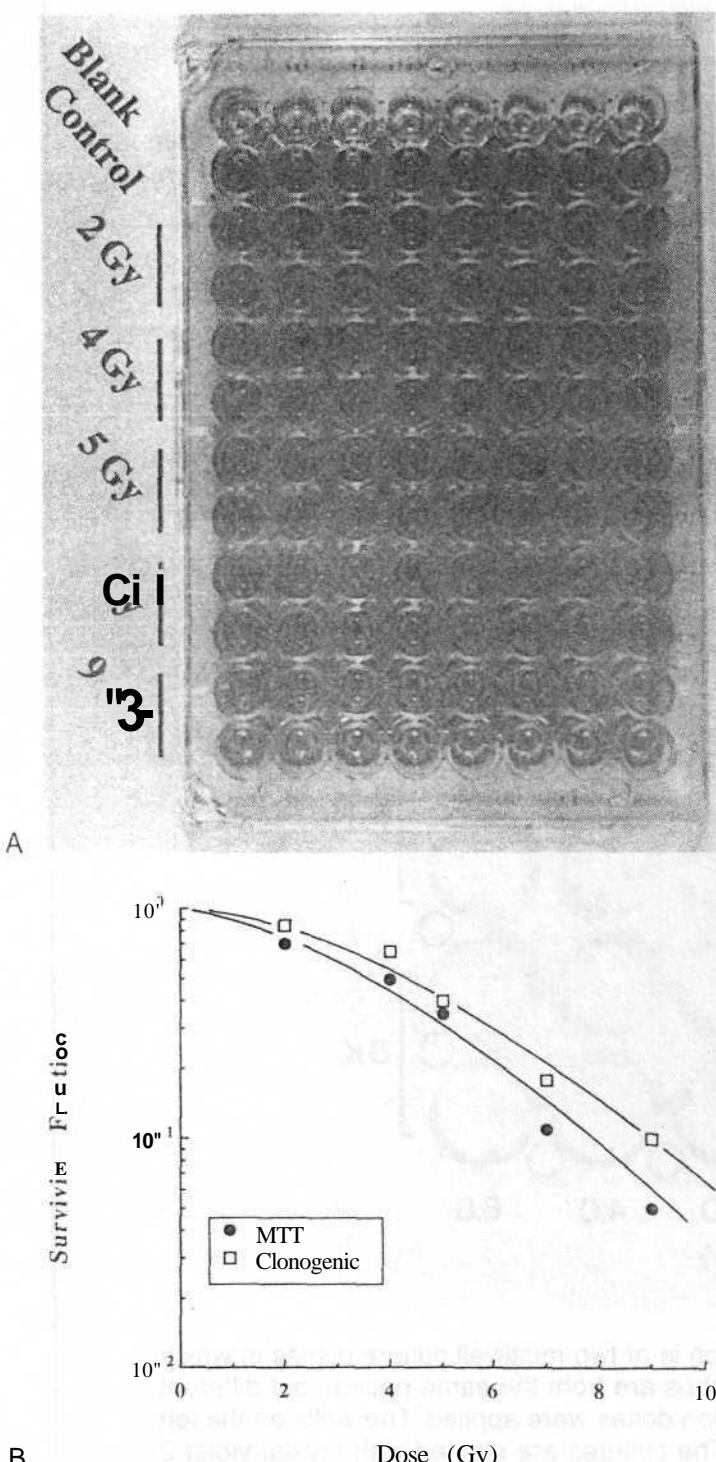
More recently, the technique has been modified to utilize dyes that can measure the total protein content or total DNA content of a well, rather than using the tetrazolium-based compound, but the principle remains the same. A large proportion of biopsy specimens grows and gives results, but a major drawback of the assay is the possible admixture of normal cells within the sample, because the tech-

nique in no way distinguishes between normal and malignant cells.

Assays of this sort are suitable for screening compounds for activity but lack the accuracy to measure SF<sub>2</sub>, or any other quantity needed in a predictive assay.

#### Cell Adhesive Matrix Assay

The basis of the cell adhesive matrix assay is to provide tumor cells from a biopsy specimen with an optimal substrate to facilitate cell attachment and growth. To accomplish this, the surface of plastic dishes is coated with a mixture of fibronectin and fibrinopeptides (CAM), and cells are grown in a special medium supplemented with hormones and growth factors. A cell suspension is prepared from a tumor biopsy specimen and known numbers of cells plated into 24-well microtest plates with a special surface, as described previously. Control and treated cells are grown for about 2 weeks, after which they are fixed and stained with crystal violet. Growth is quantitated by computerized image analysis of the stained cell monolayer in each well. The staining density of treated wells is related to that of untreated and

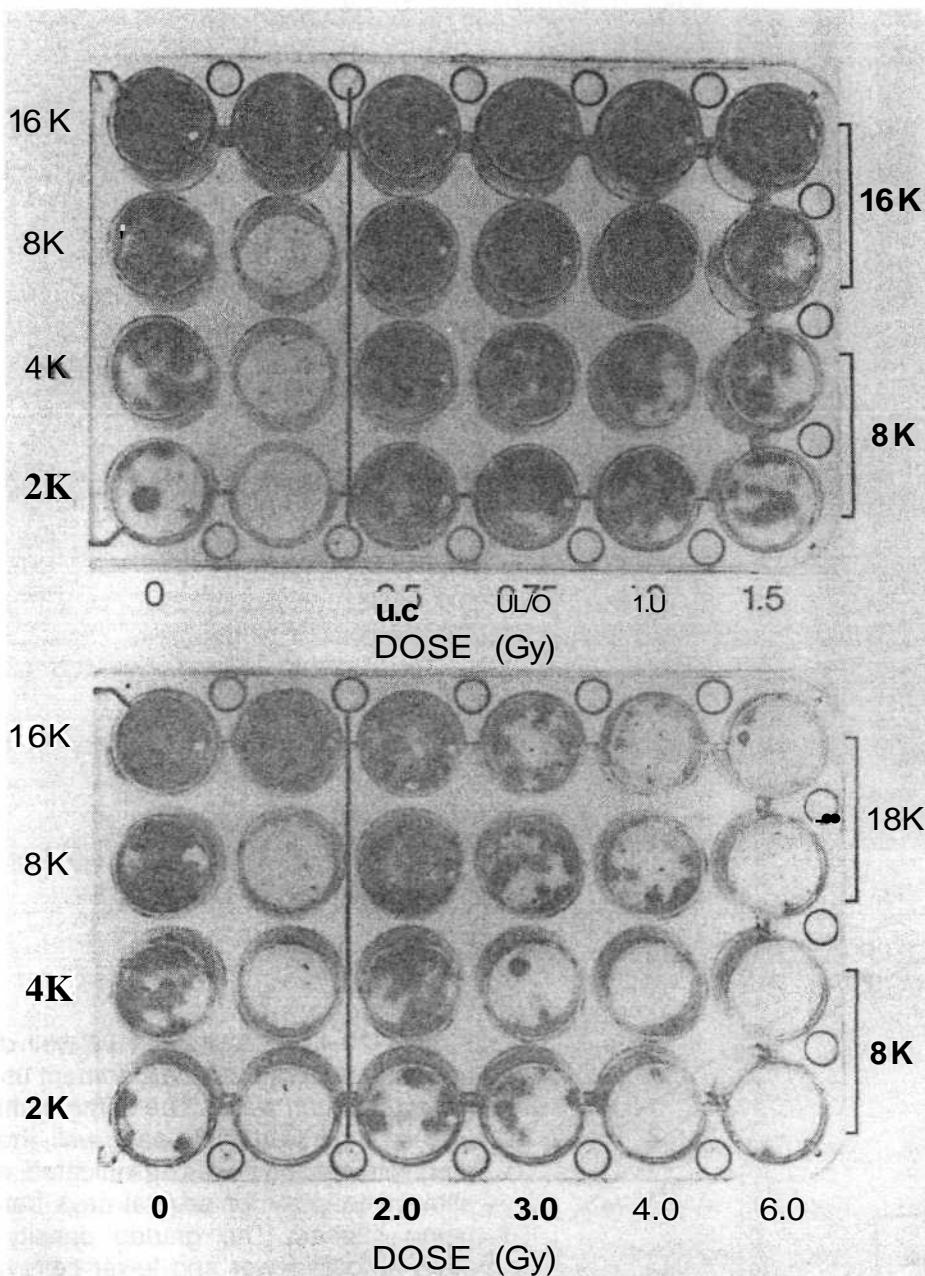


**Figure 23.4. A:** A stained 96-well dish from a typical radiation experiment using the tetrazolium assay. The same number of cells were plated into each well, irradiated with graded doses as indicated, and allowed to grow for several days before being stained. The graded density of stain reflects fewer and fewer cells surviving as the dose is increased. **B:** "Survival curve" based on spectrophotometer measurements of the dishes in A compared with a clonogenic survival curve for the same cell. (Courtesy of Dr. James Mitchell.)

a survival curve is generated. An example of plates used to establish a radiation dose-response relationship is shown in Figure 23.5. Various survival-curve parameters can be assessed, notably the SF2 (i.e., surviving fraction at 2 Gy). In common with the colonometric assays referred to earlier, this is a growth assay: It does not measure reproductive integrity, it is

limited to 1 log of killing to 10% **survival**, and its accuracy at 2 Gy is questionable.

Trials have been performed to evaluate the correlation between the pretreatment CAM assay and clinical responsiveness. Early encouraging results have not been confirmed in later studies, and the predictive usefulness of the test in individual patients is uncertain.



**Figure 23.5.** The adhesive matrix assay. The photograph is of two multiwell culture dishes in which cells from a human melanoma were grown. The two dishes are from the same patient, but different original numbers of cells were used and different radiation doses were applied. The wells on the left (marked 0) are controls with increasing cell numbers. The cultures are stained with crystal violet 2 weeks after plating, and the total amount of stain uptake, expressed as optical density, is measured by a video-image analysis system. This measurement of staining density is proportional to the number of cells that have grown in each culture well. It is clear from the figure that increasing doses of radiation result in less and less cell growth. The ratio of the density of cell growth at each radiation dose to the growth of unirradiated controls is used as a surrogate for surviving fraction. (From Brock W, Campbell H, Goepfert H, Peters LJ: Radiosensitivity testing of human tumor cell cultures: A potential method of predicting the response to radiotherapy. *Cancer Bull* 39:98-102, 1987, with permission.)

## Oxygen Status

### Labeled Nitroimidazoles

New modalities aimed at overcoming the perceived problem of hypoxic cells did not enjoy great success if applied indiscriminately across the board to large groups of patients. This was true for neutrons and hypoxic cell sensitizers, both of which were introduced initially on the premise that the curability of human tumors by x-rays was limited by the presence of hypoxic cells that are resistant to killing with x-rays. It would be desirable to have available an assay that would identify which individual patients have tumors containing hypoxic cells. A successful approach has been based on nitroimidazole labeled with a radioactive material. In regions of low oxygen tension, the nitroimidazole undergoes bioreduction and radioactive material becomes covalently linked to cellular molecules.

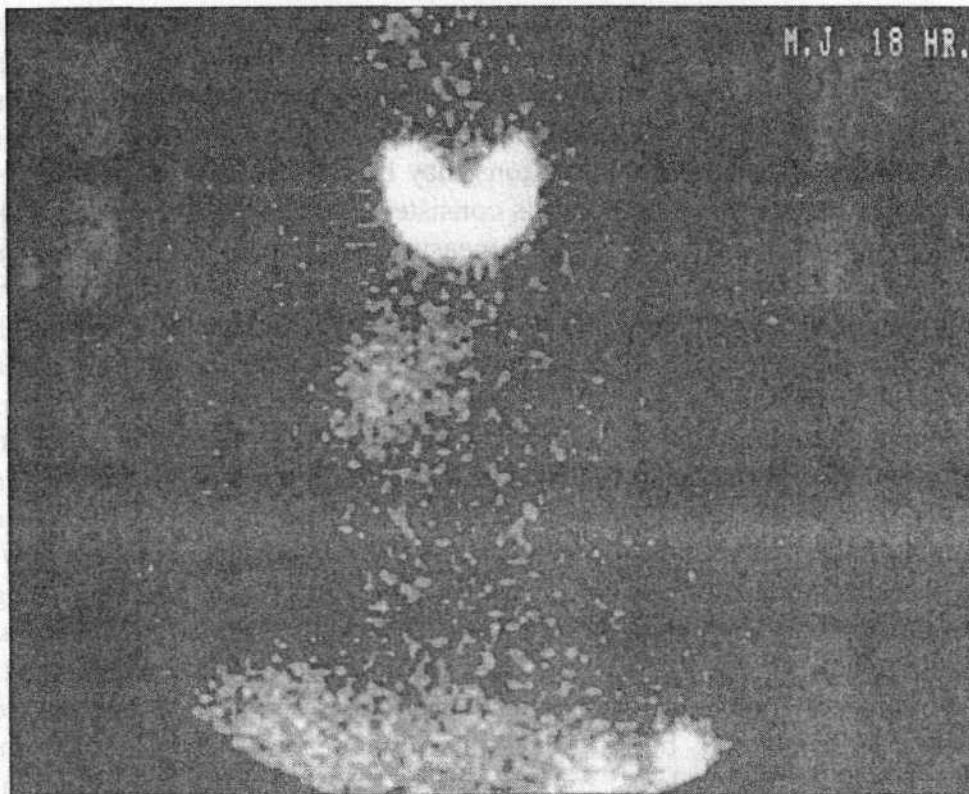
Early studies used misonidazole tagged with a (3-emitter radionuclide assayed by autoradiography. The test could not be used prospectively as a predictive assay in individual patients because (3-rays cannot be detected outside of the body, but by taking sections of tumors excised from patients previously given the labeled drug, it was shown that only a minority of tumors contain hypoxic cells.

Throughout the 1980s a considerable effort was devoted to the development of nuclear medicine markers of tissue hypoxia that could be detected by single photon emission computed tomographic (SPECT) scanning or by positron emission tomography. The research collaboration at the Cross Cancer Institute in Edmonton selected iodinated azomycin arabinoside for SPECT studies; the research group at the University of Washington in Seattle selected fluoromisonidazole for positron emission tomographic studies. Preliminary clinical investigations of iodinated azomycin arabinoside in 51 patients and fluoromisonidazole in 37 patients have been reported. There appears to be reasonable consistency between the two different approaches, and overall, between one third and one half of the tumors show significant hypoxia.

These data suggest that the oxygenation status of solid human tumors is, on average, significantly higher than that found in rodent tumors, and that their radiobiologic hypoxic fractions may be significantly lower. The result is consistent with the higher median pCb values measured for human relative to rodent tumors by the Eppendorf pCb probe. Figure 23.6 shows a SPECT image of a small-cell lung cancer 24 hours after the administration of iodinated azomycin arabinoside, which is labeled with iodine-123. The label is not attached as well as in the case of [3-emitters, and as a consequence there is uptake of released iodine in the thyroid. An hypoxic area, however, shows up clearly in the small-cell lung cancer. This represents a noninvasive test that can be performed on individual patients prospectively. The results could be used to "select" those patients likely to benefit from, for example, a hypoxic cell cytotoxin or radiosensitizer.

## Oxygen Probes

Oxygen probes, that is, electrodes implanted directly into tumors to measure oxygen concentration by a polarographic technique, have a long and checkered history. The situation has improved, however, as a consequence of technical developments of considerable interest and importance. One of the longstanding problems is that the implantation of a rigid probe crushes tissue, compresses vessels, and causes damage, thereby altering the oxygen tension, the very quantity that is being measured. The breakthrough was the development of the Eppendorf probe, a commercially available polarographic electrode that moves quickly through tissue under computer control and has a *very fast* response time of about 1 second. These properties circumvent any significant effect on the recorded local tissue pO<sub>2</sub> brought about by the compression of vessels in the vicinity of the electrode and the oxygen consumption of the cathode. The fact that the probe is rigid and not particularly small is thus largely irrelevant. The probe moves under computer con-



**Figure 23.6.** Single photon emission computed tomographic image of a human small-cell lung cancer 24 hours after administration of iodinated azomycin arabinoside, which is labeled with iodine-123. Note the "bow-tie" uptake of released iodine in the thyroid, and the uptake in the tumor (lower righthand corner), indicating the presence of hypoxic cells. (Courtesy of Dr. J. D. Chapman, Fox Chase Cancer Center.)

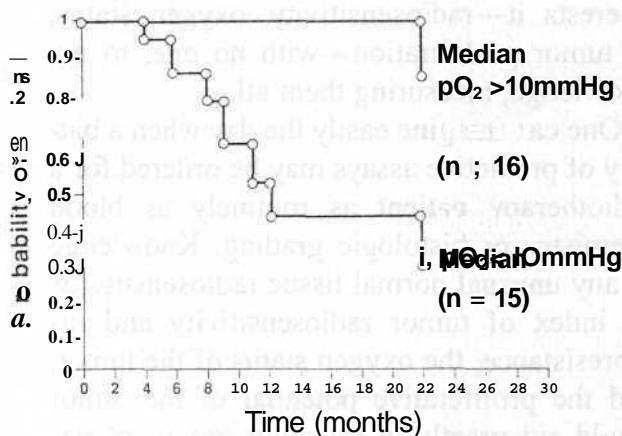
trol, making a measurement of  $p\text{Ch}$  every second as it moves along a track through the tumor in steps of about 1 mm.

A prospective clinical trial was conducted at the University of Mainz in Germany to investigate the usefulness of this new generation of oxygen probes. Patients with locally advanced carcinoma of the uterine cervix were treated with a combination of external-beam radiotherapy and three high dose rate brachytherapy insertions. Before treatment, measurements of oxygen concentration were made in each patient, 25 to 30 measurements along each of two tracks through the tumor.

Patients whose tumors exhibited median  $p\text{Ch}$  values of less than or equal to 10 mm Hg had significantly lower survival and recurrence-free survival rates compared with patients with tumors in which the  $p\text{O}_2$  was greater than 10 mm Hg. Figure 23.7 shows the data for recurrence-free survival. Other fac-

tors such as tumor stage, size, histologic grading, and treatment protocol were not significantly different between the two groups. The implication is that tumor descriptors such as size, site, or histologic grade do not predict for the presence of hypoxia. This dramatic result appears to confirm that oxygen-probe measurements represent a practical predictive assay to identify hypoxic tumors that may benefit from alternate treatment strategies such as radiosensitizers or bioreductive drugs. They compete with radiolabeled nitroimidazoles detected by SPECT imaging, although they do suffer the disadvantage that an invasive procedure is involved.

The obvious and straightforward interpretation of these data is that the presence of hypoxic cells makes tumors intransigent to cure by radiation because of the oxygen effect. A subsequent study, however, showed that survival was poorer in similar patients with low



**Figure 23.7.** Recurrence-free survival in patients with advanced carcinoma of the cervix treated by a combination of external-beam radiotherapy and high dose rate brachytherapy. The patients were divided into two groups on the basis of pretreatment oxygen-probe measurements that indicated mean  $pO_2$  values of greater than or of less than or equal to 10 mm Hg. (Adapted from Hockel M, Knoop C, Schlenger K, et al.: Intratumoral  $pO_2$  predicts survival in advanced cancer of the uterine cervix. Radiother Oncol 26:45-50, 1993, with permission.)

$pO_2$  values even if surgery was used instead of radiotherapy. The implication is that hypoxia is a measure of tumor aggression. This is discussed in more detail in the chapter on the oxygen effect (Chapter 6).

#### Proliferative Potentiation

There are more and more data accumulating that suggest overall treatment time to be a very important factor determining the outcome of head-and-neck cancer treated by radiation. This includes the CHART study, three fractions per day for 12 days; the European Cooperative Radiotherapy Group (EORTC) trial of accelerated fractionation; the DAHANCA (Danish head and neck cancer study group) study, six *versus* five fractions per week; and the Polish study of seven *versus* five fractions per week. These data strongly suggest, but do not prove conclusively, that tumor cell repopulation during treatment is an important factor determining outcome.

Strategies to achieve shorter overall treatment times, such as giving two or three frac-

tions per day, or treating Saturdays and Sundays as well as weekdays, represent inconvenient, labor-intensive, and therefore expensive forms of treatment. They should be reserved, therefore, for patients likely to benefit, and so what is required is a pretreatment cell kinetic parameter that can be measured quickly and easily to pick out fast-growing tumors.

Some years ago, it appeared that the potential tumor doubling time ( $T_{pot}$ ) was just such a parameter.  $T_{pot}$  is a measure of tumor growth that includes the cell-cycle time and the growth fraction but excludes the effect of cell loss.  $T_{pot}$  can be estimated by flow cytometry from a single tumor biopsy taken several hours after an injection of bromodeoxyuridine. The details are described further in the chapter on cell and tissue kinetics (Chapter 21).

The EORTC performed a trial to compare an accelerated protocol (72 Gy/5 weeks/3 fractions per day) with conventional therapy (70 Gy/7 weeks/1 fraction per day) in patients suffering from head-and-neck cancer. If all patients were considered together, there was no significant difference in local tumor control between the conventional and accelerated protocol. Tumors with a  $T_{pot}$  shorter than 4 days were described as "fast" growing, and those with a  $T_{pot}$  longer than 4 days were regarded as "slow" growing. It was found that local control in patients with slow-growing tumors did not benefit from the accelerated treatment protocol. By contrast, the minority of patients with fast-growing tumors ( $T_{pot}$  shorter than 4 days) showed greatly improved local tumor control on the accelerated protocol.

Later, however, a European multicenter study with more patients and a longer follow-up did not confirm this result. It appears that  $T_{pot}$  does not predict the outcome of head-and-neck cancer treated by radiotherapy. The probable reason for this is that  $T_{pot}$  is measured prospectively in the undisturbed tumor prior to treatment, whereas what determines outcome is the effective doubling time during a protracted fractionated course of radiation therapy. This same multicenter trial found that the la-

beling index or the length of the DNA synthetic phase correlated with outcome better than T<sub>pot</sub>, though the correlation was not sufficiently strong to be useful as a predictive assay.

The conclusion at the present time must be that the overwhelming evidence indicating that longer overall times lead to a worse outcome imply that tumor repopulation during treatment is a vital concern. There is, however, no robust measurable indicator of repopulation that can be used reliably as a predictive assay.

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Predictive assays are in the developmental stage; they have not yet proved their worth and certainly have not found an accepted place in routine radiotherapy. For the most part they are restricted to a few research-oriented institutions, and then a given laboratory usually specializes in the test that particularly

interests it—radiosensitivity, oxygen status, or tumor proliferation—with no one, to my knowledge, measuring them all.

One can imagine easily the day when a battery of predictive assays may be ordered for a radiotherapy patient as routinely as blood chemistry or histologic grading. Knowledge of any unusual normal tissue radiosensitivity, an index of tumor radiosensitivity and radioresistance, the oxygen status of the tumor, and the proliferative potential of the tumor would aid greatly in selecting groups of patients who might benefit from accelerated treatment, hyperfractionation, or bioreductive drugs as adjuncts to radiotherapy. In the past, the trial of new strategies in radiotherapy has been hampered significantly by the heterogeneity of patient populations. Clinical trials require greatly increased numbers of patients in each arm of the protocol if the new agent or technique benefits only a subset of the patients entered on the trial.

### SUMMARY OF PERTINENT CONCLUSIONS

- The aim of predictive assays is to identify patients whose tumors (or normal tissues) show unusual intrinsic sensitivity or resistance to radiation or whose tumors are intransigent to conventional treatment because of the presence of hypoxic cells or a rapid proliferative potential.
- It is possible that conventional protocols are designed to avoid problems in the normal tissues of a radiosensitive minority, thus underdosing the average patient.
- Pilot studies with patients on radiotherapy indicate a correlation between the radiosensitivity of normal tissue fibroblasts cultured *in vitro* and late reactions in the same patients from whom the cells were taken.
- In the future, radiosensitive subsets of patients may be identified by genetic analysis rather than by measurements of radiosensitivity from biopsy samples.
- Predictive assays for tumors fall into three categories: intrinsic radiosensitivity, oxygen status, and proliferative potential.

#### Intrinsic Radiosensitivity

- Intrinsic cellular radiosensitivity can be measured by clonogenic assays. Cell lines from human tumors can be grown by conventional culture techniques. One of the problems is that clonogenic assays take too long to realistically be used prospectively as a predictive assay.

- The initial region of the survival curve correlates best with clinical results: either the SF2, the fraction of cells surviving 2 Gy, or  $\alpha$ , the linear component or initial slope.
- West and her colleagues found a lower probability of local tumor control in patients with carcinoma of the cervix treated with radiotherapy if the SF2 (measured by the Courtenay assay) was greater than 0.55.
- Tumors have been divided into six histologic groups: From the most radioresistant to the most radiosensitive they are glioblastomas, melanomas, squamous cell carcinomas, adenocarcinomas, lymphomas, and oat-cell carcinomas. The order of SF<sub>2S</sub> correlates with clinical responsiveness, but these data come from cell lines and do not allow predictions of individual patients.
- Nonclonogenic assays have been developed based on cell growth in multiwell dishes. Growth is assessed by density of stain. These assays are rapid, easy, and amenable to automation.
- Cells from biopsy specimens of human tumors do not grow well as attached cells, using conventional techniques, but can be grown in soft agar using the Courtenay assay.
- **Colorimetric** assays such as the MTT assay, or derivatives of it, are based on the ability of viable cells to reduce a compound that can be visualized by staining or are based on total DNA or RNA content of a colony of cells growing after irradiation. These endpoints are surrogates for reproductive integrity. Such assays are suitable for screening drugs for activity but are not suitable for predictive assays.
- The cell adhesive matrix assay also is based on cell growth, not clonogenicity. It utilizes a surface specially prepared so that cells from a biopsy specimen attach and grow. Such assays have yet to be proven as predictive assays.

#### Oxygen Status

- The oxygen status of a tumor may be assessed by deposited labeled nitroimidazoles in the tumor or by polarographic oxygen probes.
- Compounds labeled with the short-lived ( $\beta$ -emitting isotope iodine-123 also can be used in regions of low oxygen tension; the compound is **covalently** linked and the isotope deposited. The presence of hypoxia can be visualized by SPECT imaging.
- Eppendorf probes have a very fast response and can be moved quickly through a tumor under computer control to obtain an oxygen profile.
- A clinical trial in Germany of patients with advanced carcinoma of the cervix treated with radiotherapy has shown lower survival and recurrence-free survival in patients whose tumors exhibited median  $pO_2$  values less than or equal to 10 mm Hg as measured by the Eppendorf probe.
- Although this evidence initially was interpreted to show that radioresistance resulting from hypoxia was important, later studies indicate that hypoxia leads to more aggressive and malignant tumors.

#### Proliferative Potential

- The proliferative potential of a tumor can be expressed in terms of the potential doubling time ( $T_{pot}$ ), which takes into account cell cycle time and growth fraction but not cell loss.
- $T_{pot}$  can be measured in individual patients by flow cytometry on a single tumor biopsy specimen taken some hours after an injection of bromodeoxyuridine.

- One clinical study by the EORTC of head-and-neck cancer shows that fast-growing tumors ( $T_{pot}$  less than 4 days) show improved local control following accelerated fractionation compared with conventional therapy, but slow-growing tumors do not. Later studies failed to find a correlation between  $T_{pot}$  and radiotherapy outcome. Labeling index and the length of the DNA phase correlate weakly with treatment outcome, but not sufficiently to be used as a predictive assay.
- There is strong evidence that lengthening overall treatment time leads to a poorer outcome of radiotherapy, at least in head-and-neck cancers. This implies that repopulation during treatment is important, but at the present time there is no robust reliable method to prospectively predict which tumors will respond poorly because of rapid repopulation.
- Predictive assays are in a developmental stage; they have not yet proved their worth but show some promise in selecting groups of patients that may benefit from altered treatment protocols: accelerated treatment, hyperfractionation, bioreductive drugs, neutrons, and so on.

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## Alternative Radiation Modalities

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### FAST NEUTRONS

#### BORON NEUTRON-CAPTURE THERAPY

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### PROTONS

#### SUMMARY OF PERTINENT CONCLUSIONS

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The early recognition that x-rays could produce local tumor control in some patients and not in others led to the notion that other forms of ionizing radiations might be superior.

Neutrons first were introduced in a speculative way, not based on any particular hypothesis. The later use of neutrons and the introduction of protons, negative  $\pi$ -mesons, and heavy ions all were based clearly on a putative advantage, either of physical dose distribution or radiobiologic properties.

The use of neutrons following World War II was based squarely on the premise that the presence of hypoxic cells limits the curability of human tumors by x-ray therapy, so that the lower oxygen enhancement ratio (OER) characteristic of neutrons might confer an advantage. An alternative rationale for neutrons, proposed at a later date, was that their relative biologic effectiveness (**RBE**) is larger for slow-growing tumors, so that they may have an advantage in a limited number of specific human tumors.

Protons have radiobiologic properties similar to x-rays, and their introduction into radiotherapy was based entirely on the superiority of the physical dose distribution possible with charged particles. Negative  $K^-$  mesons and heavy ions were introduced with the hope of combining the radiobiologic advantages attributed to neutrons with the dose distribution advantage characteristic of protons.

Neutrons have been shown to be superior to x-rays in a limited number of situations, specifically for the treatment of prostatic cancer and salivary gland tumors. A number of controlled clinical trials have been **performed** for a wide variety of cancer sites, but a gain was apparent only in these few circumstances. Protons have found a small but important niche for the treatment of uveal melanoma and **tumors** such as chordomas that are located close to the spinal cord and therefore benefit greatly from the localized dose distribution. The wider use of protons for broad-beam radiotherapy is being tested, but no advantage has been proven yet. Negative  $n$ -mesons and heavy ions have been used to treat hundreds of patients, but prospective randomized trials have never been completed to prove their superiority over conventional x-rays. Their **enormous** cost would be justified only by a significant gain.

The casual reader may be content with this overview of alternative radiation modalities and may not wish to proceed further in this chapter. Interest in high linear energy transfer (LET) radiations for radiotherapy largely has waned, but protons are very much in **vogue**. In this chapter neutrons and protons are considered in turn.

### FAST NEUTRONS

#### Rationale

Neutrons first were used for cancer therapy at the Lawrence Berkeley Laboratory in Cali-

formia in the 1930s. Their use was not based on any biologic or physical rationale; they were used only because they represented a new modality that might be useful in hopeless cancer cases, for which conventional radiations were known to be ineffective.

After World War II, interest in neutrons for cancer treatment was renewed at the Hammersmith Hospital in London, as a result of studies that implied that tumors contain hypoxic cells and that cells deficient in oxygen are resistant to killing by x-rays. The rationale for neutrons at this stage, therefore, was their lower dependence on oxygen for cell killing, together **with** the premise that viable hypoxic cells limit curability by x-rays.

Clinical trials to date have shown clearly that neutrons do not offer an advantage over **x-rays** across the board for a broad spectrum of tumor types. Nevertheless, there is tantalizing evidence that they give better results for certain types of tumors. This, together with other evidence, has resulted in a rethinking of the role of hypoxic cells and the admission that they probably are not as important as previously thought, at least in multifraction regimens in which reoxygenation can be effective. The revised rationale for neutrons, therefore, is that RJBE varies for different tumor types, being high for some that are slowly proliferating. On this basis neutrons would be expected to offer an advantage only in a few selected types of cases. The idea is that slowly growing, well-differentiated tumors may be analogous to the slowly proliferating tissues responsible for late effects, and it is well documented that neutron RBEs are higher for late than for early effects, at least for treatment schedules involving many fractions.

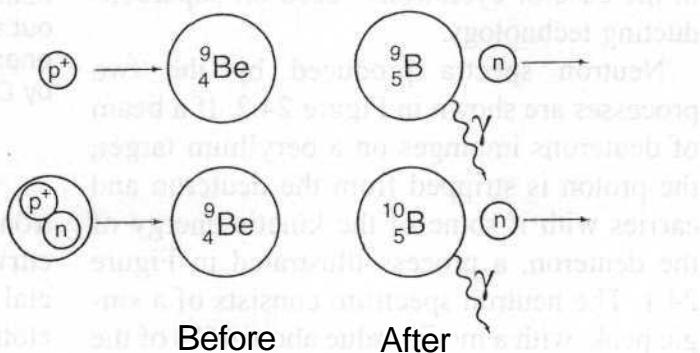
The rationale for the use of neutrons has undergone a considerable evolution over the years. The biologic properties of neutrons differ from those of x-rays in a number of respects, and it is not clear which is the most important in a clinical situation.

### Practical Sources

The only practical source of neutrons for clinical radiotherapy is a cyclotron. A cy-

clotron is an electric device capable of accelerating positively charged particles, such as protons or deuterons, to an energy of millions of volts. The particle is accelerated by being made to pass repeatedly through an electric gradient while being held in a circular orbit by a magnetic field produced by a huge magnet. The path of the particle, as it accelerates, is a spiral, until it is extracted from the machine. The principle of the cyclotron was conceived by Ernest Lawrence at the University of California at Berkeley in 1931, when he realized that the time taken for a charged particle to complete a circular orbit in magnetic field was independent of the radius of the orbit.

Neutrons can be produced in a cyclotron by accelerating deuterons or protons and making them impinge on a beryllium target (Fig. 24.1). Using the  $d^+ \rightarrow Be$  process, a high yield of neutrons is readily achievable; the disadvantage is that the cyclotrons needed are relatively massive. Early on, a few low-energy machines, of 15 MeV or less, were built specially for medical use and installed in hospitals, with their time divided

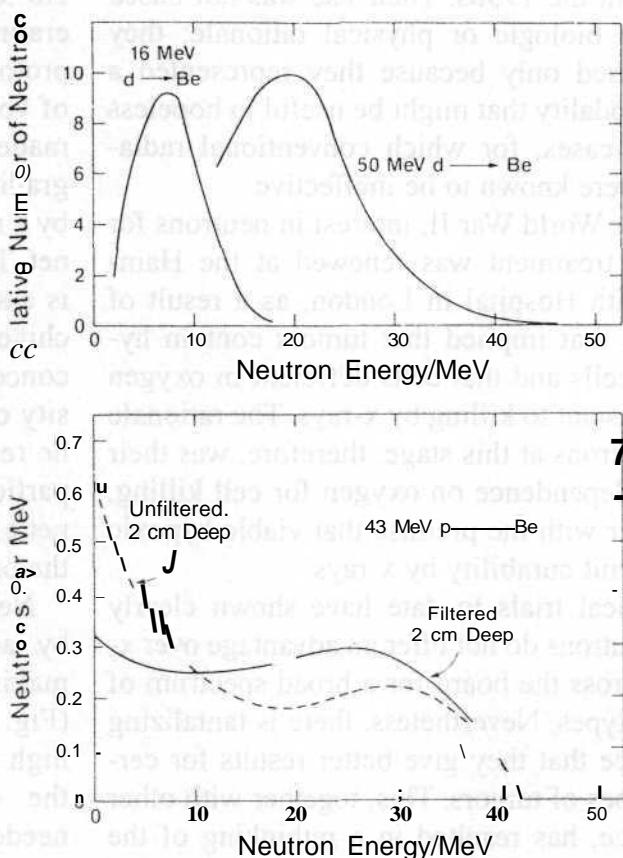


**Figure 24.1.** Diagram illustrating the two neutron-production processes in common usage. **Top:** The  $p^+ \rightarrow Be$  process. Protons are accelerated to high energy in a cyclotron and made to impinge on a target of beryllium, where they "knock out" neutrons. **Bottom:** The  $d^+ \rightarrow Be$  stripping process. Deuterons are accelerated to high energy in the cyclotron and are then made to impinge on a beryllium target. When incident on the target, the proton is "stripped" from the deuteron, leaving a neutron that retains part of the incident kinetic energy of the accelerated deuteron. For each neutron produced, one atom of beryllium is converted to boron.

between neutron cancer therapy and the production of short-lived positron-emitting radionuclides. This was the policy in Great Britain, with neutron therapy being administered with small cyclotrons using the  $d^+ \rightarrow Be$  process at Hammersmith and Edinburgh. Unfortunately, the limited energy from this process results in poor percentage depth doses, and only relatively superficial tumors in the head and neck could be treated adequately. To obtain penetration comparable to megavoltage x-rays using neutrons produced by the  $d^+ \rightarrow Be$  process requires an accelerating energy of about 50 MeV, and that means a massive cyclotron, much too large to be accommodated in a hospital. In the United States several high-energy machines (22-50 MeV), initially designed and built at enormous cost for high-energy physics research, were modified and used to generate neutron beams for cancer therapy on a part-time basis.

More recently, cyclotrons to produce neutrons have been built using the  $p^+ \rightarrow Be$  reaction. Because a proton has half the mass of a deuteron, the cyclotron can be sufficiently small to be installed in a hospital, particularly in the case of cyclotrons based on superconducting technology.

Neutron spectra produced by the two processes are shown in Figure 24.2. If a beam of deuterons impinges on a beryllium target, the proton is stripped from the deuteron and carries with it some of the kinetic energy of the deuteron, a process illustrated in Figure 24.1. The neutron spectrum consists of a single peak, with a modal value about 40% of the energy of the incident deuterons. Thus, 50-MeV deuterons would produce a neutron beam with a modal energy of about 20 MeV [if accelerated protons impinge on a beryllium target, neutrons are produced by a knock-on process, and the neutron spectrum spans a wide range (Fig. 24.1)]. Many low-energy neutrons are produced, as well as neutrons up to energies close to the accelerating energy of the incident protons. In many cases it is necessary to use a filter of some hydrogenous material, such as polyethylene, to filter out preferentially some of the low-energy neu-



**Figure 24.2.** Neutron spectra resulting from (top) cyclotron-produced neutrons by the  $d^+ \rightarrow Be$  process for the highest and lowest energies used clinically, and (bottom) cyclotron-produced neutrons by the  $p^+ \rightarrow Be$  process, with and without a hydrogenous filter to remove the "soft" low-energy neutrons. (Compiled from data published by Dr. Paul Kliauga.)

trons that would "spoil"<sup>7</sup> the depth-dose curves because they are absorbed at superficial depths (Fig. 24.2). A 50-MeV proton cyclotron produces neutrons with depth doses similar to a deuteron cyclotron of the same or slightly higher energy and is a fraction of its size. Dedicated hospital-based cyclotrons in the 50- to 70-MeV range using the  $p^+ \rightarrow Be$  reaction are used in neutron cancer therapy in a number of countries. Such machines can be built with an isocentric mount and adjustable multileaf collimators. The resultant depth doses are comparable to a 6-MV x-ray Linac. Controlled clinical trials to compare neutrons with x-rays now can be performed without the neutrons being at a disadvantage because of poor physical characteristics.

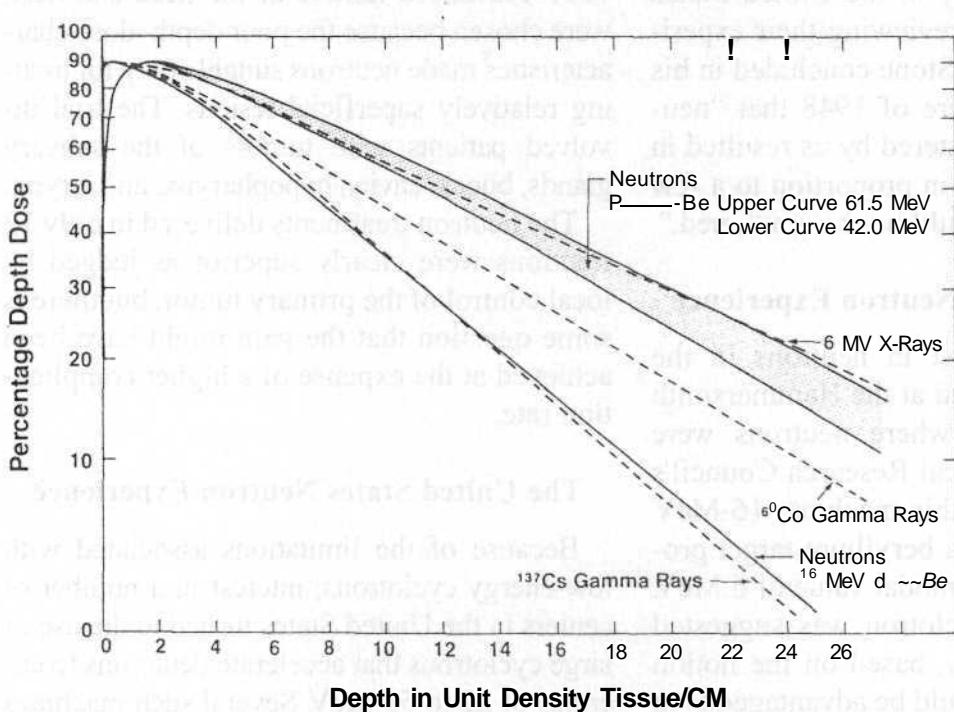
### Percentage Depth Doses for Neutron Beams

An essential factor in the choice of a neutron beam for clinical use is its ability to penetrate to a sufficient depth. Figure 24.3 is a comparison of the percentage depth doses for various photon beams with those for neutrons produced by cyclotrons using the  $d^+ \rightarrow Be$  or  $p^+ \rightarrow Be$  processes. The lower-energy Hammersmith cyclotron, used in the early trials, gave appreciably poorer depth-dose characteristics and is in fact comparable to a cesium-137 unit. The higher-energy cyclotrons show considerably better penetration. The depth doses associated with a 50-MeV cyclotron using the  $d^+ \rightarrow Be$  process or high-energy cyclotrons using the  $p^+ \rightarrow Be$  process rival those of a linear accelerator in the 4- to 6-MeV range. The acceptable depth doses associated with neutron machines are to some extent a function of the long treatment distances used, which are usually 100 to 140 cm. This distance is necessitated by the collimator, which must be thick because it is made of a hydrogenous

material to absorb the neutrons and a metal such as lead to remove the y-ray component.

### The First Clinical Use of Neutrons

The first clinical trial of neutrons was not based on any radiobiologic rationale but was prompted largely by the availability of a new and unique beam (Fig. 24.4). It is said that it received some impetus when the mother of the Lawrence brothers (E. O. Lawrence was the inventor of the cyclotron and the director of what was to become the Lawrence Berkeley Laboratory) contracted cancer, which was judged by her physician to be incurable by conventional means. She was treated with neutrons and lived for many years, although from a retrospective review of the case it is probable that she did not have cancer in the first place. This early effort at Berkeley was hampered because the complexities of the relationship between RBE and dose for high-LET radiations were not understood at the time. Consequently, a number of patients were overdosed seriously before the trial was



**Figure 24.3.** A comparison of the percentage depth doses for selected neutron beams with x- and  $\gamma$ -rays. Neutrons generated by 16-MeV  $d^+ \rightarrow Be$  have poor depth doses, comparable to a cesium-137 unit with a short source-skin distance. To obtain depth doses comparable to megavoltage photon beams requires about 50 MeV, using either the  $d^+ \rightarrow Be$  or the  $p^+ \rightarrow Be$  reaction. A cyclotron to accelerate protons to this energy is much smaller and can be accommodated in a hospital. (Compiled from data published by Dr. Paul Kliauga.)



**Figure 24.4.** The first patient treated with neutrons at the Lawrence Berkeley Laboratory of the University of California. On the left is Dr. Robert Stone, the radiotherapist, and in the center is Dr. John Lawrence, the physician brother of the inventor of the cyclotron, E. O. Lawrence. (Courtesy of the University of California.)

terminated by the entry of the United States into World War II. In reviewing their experience many years later, Stone concluded in his famous Janeway Lecture of 1948 that "neutron therapy as administered by us resulted in such bad late sequelae in proportion to a few good results that it should not be continued."

#### The Hammersmith Neutron Experience

The renewed interest in neutrons in the postwar years originated at the Hammersmith Hospital in London, where neutrons were generated by the Medical Research Council's 60-inch cyclotron. In this machine, 16-MeV deuterons incident on a beryllium target produced neutrons with a modal value of 6 MeV. The Hammersmith cyclotron was suggested and conceived by Gray, based on the notion that a lowered OER would be advantageous to radiotherapy. The machine suffered from the limitations of poor depth doses (equivalent to 250 kVp x-rays) and a fixed horizontal beam.

A prospective randomized clinical trial to compare neutrons with x-rays was started in

1971. Advanced tumors of the head and neck were chosen because the poor depth-dose characteristics made neutrons suitable only for treating relatively superficial lesions. The trial involved patients with tumors of the salivary glands, buccal cavity, hypopharynx, and larynx.

The neutron treatments delivered in only 12 fractions were clearly superior as judged by local control of the primary tumor, but there is some question that the gain might have been achieved at the expense of a higher complication rate.

#### The United States Neutron Experience

Because of the limitations associated with low-energy cyclotrons, interest in a number of centers in the United States turned to the use of large cyclotrons that accelerate deuterons to energies of 22 to 50 MeV. Several such machines were built for high-energy physics research and were converted for part-time neutron therapy. In addition, neutrons were produced at the Fermilab in Batavia, Illinois, by bombarding a beryllium target with 67-MeV protons.

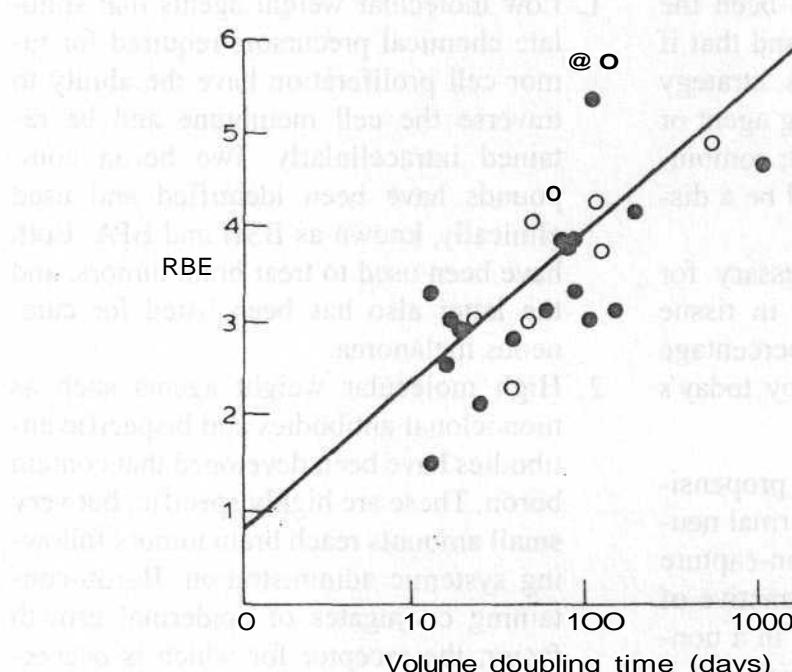
The early U.S. neutron therapy experience was accumulated in these four facilities. All had adequate dose rates and quite good depth doses. Unfortunately, they had disadvantages: All machines had fixed horizontal beams, and all were located in physics installations rather than in large busy hospitals, so that the availability of a sufficient number of patients was a problem. A number of controlled clinical trials were performed for a variety of tumor sites and showed no advantage for neutrons over x-rays. Neutrons, however, appeared to be superior for salivary gland tumors, soft-tissue sarcomas, and prostate cancer.

### Current Efforts with Neutrons

Enthusiasm for neutron therapy certainly has waned just at a time at which technology allows machines to be built that are suitable for clinical use. The new generation of hospital-based cyclotrons, using the  $p^{\alpha} \rightarrow Be$  reaction has adequate dose rates, good percentage depth doses, and a full isocentric mount, similar to a conventional Linac. A few centers operate such machines in the United States, Eu-

rope, and Japan. A fair test now should be possible of neutrons compared with x-rays without the high-LET radiation being handicapped from the outset by limitations of access or physical specifications. Emphasis will be placed on two factors. First, subgroups of patients with specific types of tumors that may benefit from neutrons must be found. It is already reasonably clear that there is not an across-the-board benefit from neutrons, which was expected, perhaps naively, in the early days. Second, different fractionation patterns will be tried for neutrons. There is no *a priori* reason to expect that the best or most effective fractionation pattern and overall time are the same for neutrons as for x-rays. Indeed, the contrary is likely to be the case, and different fractionation regimens will be tried, especially smaller numbers of fractions in a shorter time (*i.e.*, accelerated treatment).

Emphasis will be placed on slowly growing tumors, in view of the observation of Breuer and Batterman that neutron RBE, measured from pulmonary metastases in patients, increases as tumor volume doubling time increases (Fig. 24.5). This coincides with the



**Figure 24.5.** Values of relative biological effectiveness (RBE) relative to cobalt-60 7-rays for volume changes of pulmonary metastases in patients as a function of the volume doubling time. Dots indicate the measured RBE values; open circles are estimated values if only neutron irradiation was given. (From Batterman JJ: Clinical Application of Fast Neutrons: The Amsterdam Experience, p 43. Amsterdam, Rodipi, 1981, with permission.)

clinical experience that neutrons appear to be superior for prostate and salivary-gland tumors and soft-tissue sarcoma—all relatively slowly growing tumors.

### BORON NEUTRON-CAPTURE THERAPY

The basic idea behind boron neutron-capture therapy (BNCT) is elegant in its simplicity. It has appealed to physicians, and particularly to physicists, for the best part of half a century. The idea is to deliver to the cancer patient a boron-containing drug that is taken up only in tumor cells and then to expose the patient to a beam of low-energy (thermal) neutrons that themselves produce little radiobiologic effect but that interact with the boron to produce short-range, densely ionizing  $\alpha$ -particles. Thus, the tumor is intensely irradiated, but the normal tissues are spared. There are two problems inherent in this idea that have so far proved to be intractable:

1. How does one find a "magic" drug that can distinguish malignant cells from normal cells? (The skeptic might add that searching for such a drug has been the Holy Grail of cancer research and that if one were found, the obvious strategy would be to attach an alkylating agent or an  $\alpha$ -emitting radionuclide to it; combining its use with neutrons would be a distant third.)
2. The low-energy neutrons necessary for BNCT are poorly penetrating in tissue and consequently result in percentage depth doses that are horrible by today's standards.

A number of nuclides have high propensities for absorbing low-energy or thermal neutrons; that is, they have a high neutron-capture cross-section. Boron is the most attractive of these because it is readily available in a non-radioactive form, its chemistry is such that it can be incorporated into a wide variety of compounds, and if it interacts with low-energy neutrons it emits short-range, high-LET  $\alpha$ -particles.

For BNCT to be successful, the compounds to be used should have high specificity for malignant cells, with concomitantly low concentrations in adjacent normal tissues and in blood. This, of course, is a tall order.

In the early days, the compounds used were not specially synthesized for BNCT but were already available. In the brain, which is the site for which BNCT largely has been used, some selectivity is obtained because compounds do not penetrate normal brain tissue to the same degree as brain tumors in which the blood-brain barrier is absent or severely compromised.

### Boron Compounds

Critical to the success of BNCT is the requirement that boron compounds be developed that target tumor *versus* normal cells selectively, achieve a sufficient concentration within the tumor, and produce tumor to normal tissue ratios of 3 or 4 to 1. This, of course, is a tall order.

Two classes of compounds have been proposed:

1. Low molecular weight agents that simulate chemical precursors required for tumor cell proliferation have the ability to traverse the cell membrane and be retained intracellularly. Two boron compounds have been identified and used clinically, known as BSH and BPA. Both have been used to treat brain tumors, and the latter also has been listed for cutaneous melanoma.
2. High molecular weight agents such as monoclonal antibodies and bispecific antibodies have been developed that contain boron. These are highly specific, but very small amounts reach brain tumors following systemic administration. Boron-containing conjugates of epidermal growth factor, the receptor for which is overexpressed on some tumors including glioblastoma, also have been developed.

If the blood-brain barrier is disrupted temporarily, these high molecular weight com-

pounds may have some utility, or direct intracerebral delivery may be required. They have not yet proved to be effective in clinical use.

### Neutron Sources

During fission within the core of a nuclear reactor, neutrons are "born" that have a wide range of energies. Neutron beams can be extracted from the reactor by the application of suitable techniques and the use of appropriate moderators. Thermal neutrons, or room-temperature neutrons (0.025 eV), react best with boron to produce densely ionizing particles. Unfortunately, thermal neutrons are attenuated rapidly by tissue; the half-value layer is only about 15 cm. Consequently, it is not possible to treat to depths of more than a few centimeters without heavily irradiating surface normal tissues. Nevertheless, most clinical trials in Japan have utilized neutrons of this energy.

Current interest in the United States focuses on the use of epithermal neutron beams (1-10,000 eV), which have a somewhat greater depth of penetration. These can be obtained by using moderators or filters to slow the fast neutrons into the epithermal range and filtering out the residual thermal neutrons. These epithermal neutrons do not themselves interact with the boron but are degraded to become thermal neutrons in the tissue by collisions with hydrogen atoms. Even so, the peak in dose occurs at a depth of only 2 to 3 cm, with a rapid fall-off beyond this depth. Thus, the very high surface doses are avoided but the depth doses are still poor.

The need for a nuclear reactor as a source of neutrons is a serious limitation and would preclude BNCT facilities in densely populated urban areas. If BNCT were shown to have a clear therapeutic advantage, then it would be essential to design and build compact proton accelerators as a source of neutrons. Some research has been performed in this area, and it is clear that appropriate accelerators could be produced commercially if the demand were there.

### Clinical Trials

A number of clinical trials have been performed over the years, beginning in the 1950s and 1960s. Results are tantalizing but never definitive. In more recent years, a number of patients have been treated with BNCT in the United States, but the results are largely anecdotal. The concept of BNCT is as attractive as ever, but it continues to be difficult to convert into a practical treatment modality, even for shallow tumors.

### PROTONS

Protons are attractive for radiotherapy because of their physical dose distribution; their radiobiologic properties are unremarkable. The RBE of protons is indistinguishable from that of 250-kV x-rays, which means that they are 10 to 15% more effective than cobalt-60 rays or megavoltage x-rays generated by a linear accelerator. The OER for protons also is indistinguishable from that for x-rays, namely 2.5 to 3. These biologic properties are consistent with the physical characteristics of high-energy proton beams; they are sparsely ionizing, except for a very short region at the end of the particles' range, just before they stop. In the entrance plateau the average LET is about 0.5 keV/um, rising to a maximum of 100 keV/um over a few microns as the particles come to rest. This high-LET component is restricted, however, to such a tiny length of track, and represents such a small proportion of the energy deposited, that for high-energy protons it does not have any significant effect.

The dose deposited by a beam of monoenergetic protons increases slowly with depth but reaches a sharp maximum near the end of the particles' range **in the Bragg peak**. The beam has sharp edges, **with** little side-scatter, and the dose falls to zero after the Bragg peak, at the end of the particles' range. The possibility of precisely confining the high-dose region to the tumor volume and minimizing the dose to surrounding normal tissue is obviously attractive to the radiotherapist. Protons and helium ions come closest to realizing this dream at modest cost.

Proton beams ranging in energy from 150 to 200 MeV are of interest in radiotherapy, because this corresponds to a range in tissue of 16 to 26 cm. Intense proton beams in this energy range are produced readily by cyclotrons, many of which were built initially for high-energy physics research.

Figure 24.6 shows the depth-dose curve for the 187-MeV proton beam from the synchrocyclotron at Uppsala, Sweden. The sharply defined Bragg peak occurs at a depth in tissue that depends on the initial energy of the particles.

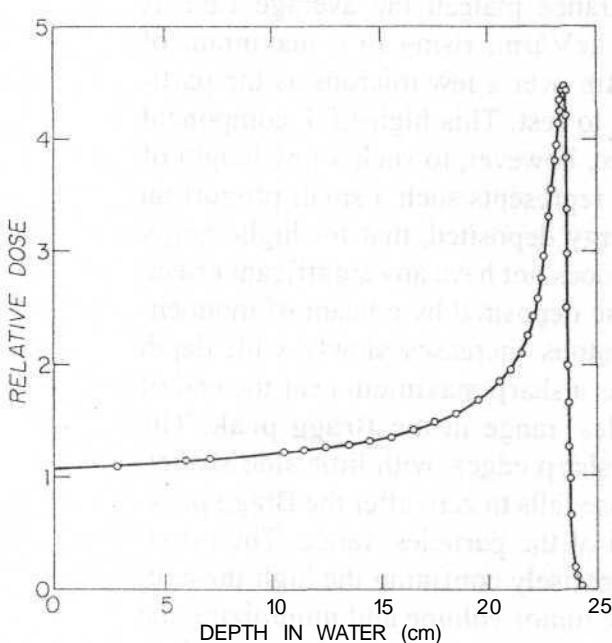
The early medical use of proton beams involved treatment of the pituitary, first in patients with advanced breast cancer and later in patients with diabetic retinopathy, Cushing's disease, and acromegaly. Protons were used for these applications to exploit their well-defined beam, which made it possible to give a huge dose to the pituitary without causing unacceptable damage to nearby structures. These treatments have been performed at both Berkeley and Harvard, although the two institutions adopted very different strategies. At Harvard, an attempt was

made to use a narrow pencil beam of protons of just the right range for the Bragg peak to fall exactly in the pituitary; in this way a huge local dose could be delivered to the gland with minimal irradiation of surrounding tissues. This would appear to be a very elegant approach to the problem, but it is fraught with difficulty because the exact location of the Bragg peak can vary considerably with small inhomogeneities in the tissue traversed. For this reason the Berkeley group favored the use of the plateau portion of a very high-energy beam that passed right through the patient's head; the Bragg peak was not within the patient at all. Multiple beams then were used in a pseudorotation technique, converging on the pituitary, to obtain good dose localization.

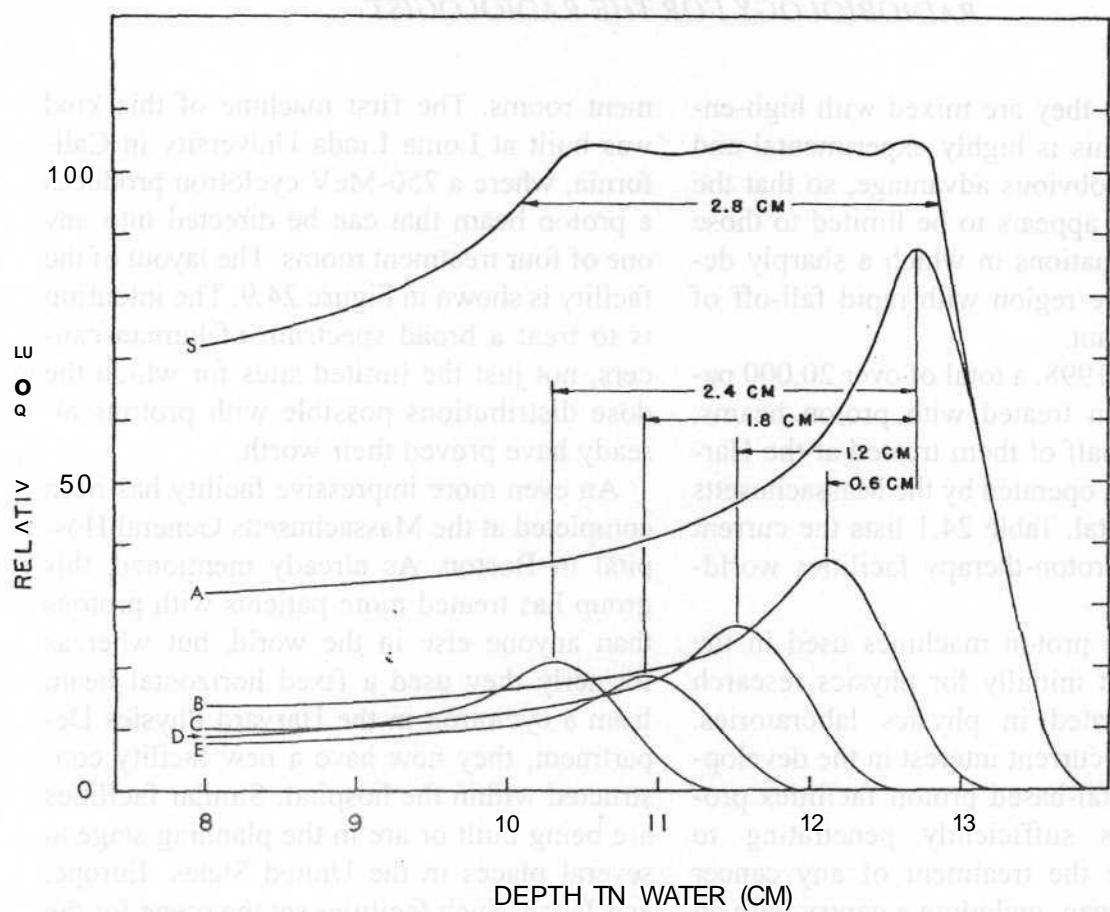
The way in which the Bragg peak can be spread out to encompass a tumor of realistic size is illustrated in Figure 24.7. In this figure, curve A shows the narrow Bragg peak of the primary beam of the 160-MeV proton beam at the Harvard cyclotron. Beams of lower intensity and shorter range, shown in curves B, C, D, and E, are readily obtainable by passing the beam through a rotating wheel with plastic sectors of varying thickness. The composite curve, S, which is the sum of all the individual Bragg peaks of the beams of varying range, results in a uniform dose over 2.8 cm. The spread-out Bragg peak, of course, can be made narrower or broader than this, as necessary.

Many researchers consider protons to be the treatment of choice for choroidal melanoma. Figure 24.8 shows the dose distribution that is achieved at the Harvard cyclotron, which allows very high doses to be delivered to small tumors without unacceptable damage to nearby normal tissues. Protons have found a small but important place in the treatment of ocular tumors and also some specialized tumors close to the spinal cord.

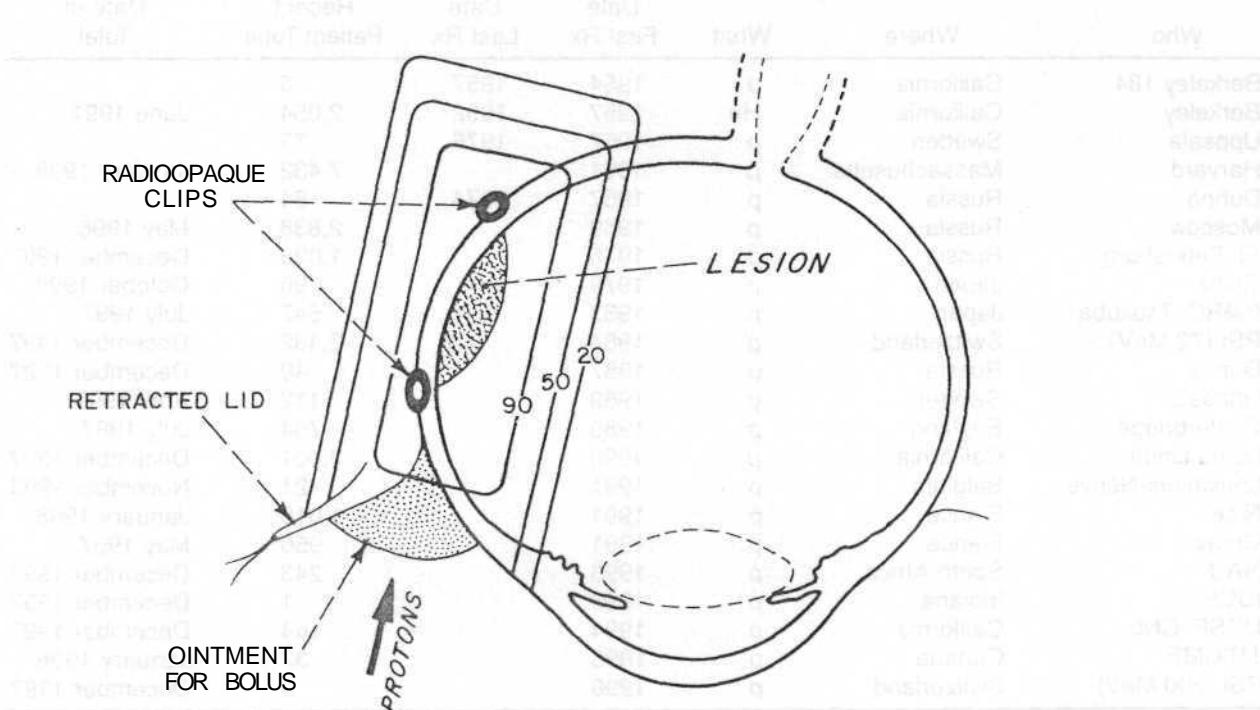
Broad-beam radiotherapy, with the Bragg peak spread out to cover a large tumor, has been in progress at Uppsala since 1957, and a comparable U.S. effort has begun at Harvard. Protons seldom are used alone in such applications because there is no skin-sparing ef-



**Figure 24.6.** Depth-dose curve for 187-MeV protons from the Uppsala synchrocyclotron. The dose reaches a sharp peak at a depth of about 23 cm. (Adapted from Larsson B: Br J Radiol 34: 143-151, 1961, with permission.)



**Figure 24.7.** The way in which the Bragg peak for a proton beam can be spread out. Curve A is the depth-dose distribution for the primary beam of 160-MeV protons at the Harvard cyclotron, which has a half-width of only 0.6 cm. Beams of lower intensity and shorter range, as illustrated by curves B, C, D, and E, can be added to give a composite curve S, which results in a uniform dose over 2.8 cm. The broadening of the peak is achieved by passing the beam through a rotating wheel with sectors of varying thickness. (Adapted from Koehler AM, Preston WM: Radiology 104:191-195, 1972, with permission.)



**Figure 24.8.** Dose distribution used for the treatment of choroidal melanoma at the Harvard cyclotron. Note the sharp edges to the beam and rapid falloff of dose outside the treatment volume. (Courtesy of Dr. Herman Suit.)

feet, but rather they are mixed with high-energy x-rays. This is highly experimental and has shown no obvious advantage, so that the use of protons appears to be limited to those specialized situations in which a sharply defined high-dose region with rapid fall-off of dose is important.

By January 1998, a total of over 20,000 patients had been treated with proton beams, with close to half of them treated at the Harvard cyclotron, operated by the Massachusetts General Hospital. Table 24.1 lists the current and planned proton-therapy facilities worldwide.

Most of the proton machines used in the past were built initially for physics research and were located in physics laboratories. There is much current interest in the development of hospital-based proton facilities producing beams sufficiently penetrating to make possible the treatment of any cancer sites in the human, including a gantry with an isocentric mount, and feeding several treat-

ment rooms. The first machine of this kind was built at Loma Linda University in California, where a 250-MeV cyclotron produces a proton beam that can be directed into any one of four treatment rooms. The layout of the facility is shown in Figure 24.9. The intention is to treat a broad spectrum of human cancers, not just the limited sites for which the dose distributions possible with protons already have proved their worth.

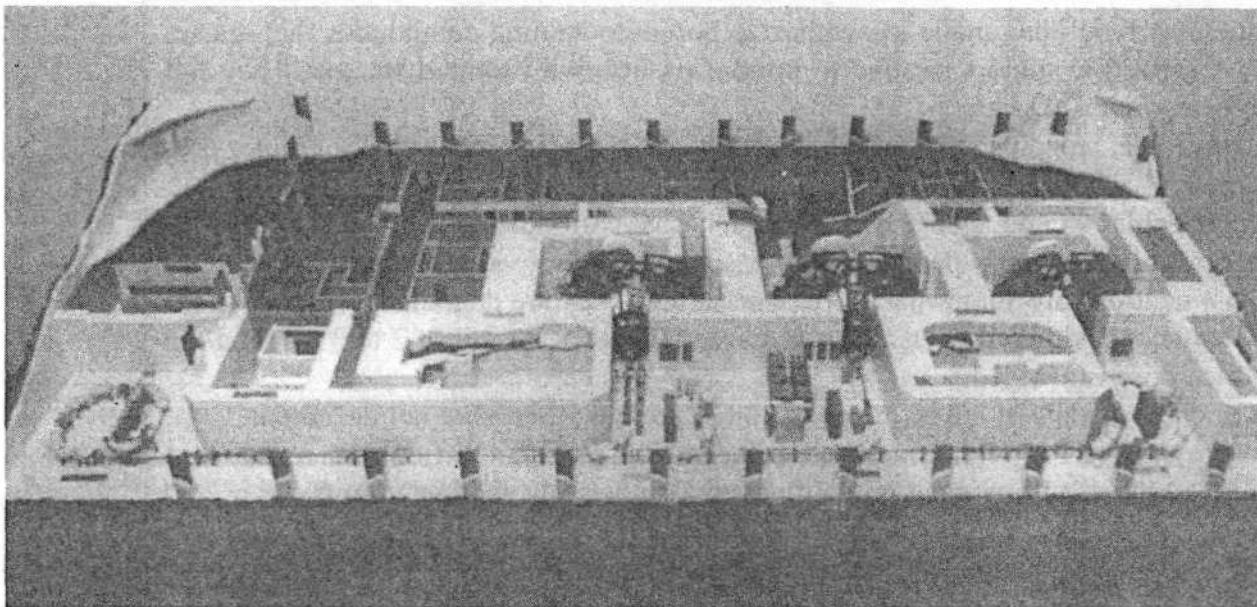
An even more impressive facility has been completed at the Massachusetts General Hospital in Boston. As already mentioned, this group has treated more patients with protons than anyone else in the world, but whereas formerly they used a fixed horizontal beam from a cyclotron in the Harvard Physics Department, they now have a new facility constructed within the hospital. Similar facilities are being built or are in the planning stage at several places in the United States, Europe, and Japan. Such facilities set the scene for the future.

TABLE 24.1. Worldwide Proton Facilities and Patient Totals (as of January 1998)

Who	Where	What	Date First Rx	Date Last Rx	Recent Patient Total	Date of Total
Berkeley 184	California	P	1954	1957	3	
Berkeley	California	He	1957	1992	2,054	June 1991
Uppsala	Sweden	P	1957	1976	73	
Harvard	Massachusetts	P	1961		7,432	January 1998
Dubna	Russia	P	1967	1974	84	
Moscow	Russia	P	1969		2,838	May 1996
St. Petersburg	Russia	P	1975		1,028	December 1997
Chiba	Japan	P	1979		96	October 1996
PMRC, Tsukuba	Japan	P	1983		547	July 1997
PSI (72 MeV)	Switzerland	P	1984		2,482	December 1997
Dubna	Russia	P	1987		40	December 1997
Uppsala	Sweden	P	1989		112	April 1997
Clatterbridge	England	P	1989		764	July 1997
Loma Linda	California	P	1990		3,001	December 1997
Louvain-la-Neuve	Belgium	P	1991		21	November 1993
Nice	France	P	1991		1,010	January 1998
Orsay	France	P	1991		956	May 1997
NAC	South Africa	p	1993		243	December 1997
IUCF	Indiana	P	1993		1	December 1997
UCSF-CNL	California	P	1994		144	December 1997
TRIUMF	Canada	P	1995		37	January 1998
PSI (200 MeV)	Switzerland	P	1996		9	December 1997

Based on *Particles*, a newsletter sponsored by the Proton Therapy Cooperative Group.

Rx, Radiotherapy; p, proton; He, helium ion



**Figure 24.9.** Model of the proton facility at Loma Linda. Protons are accelerated to energies up to 250 MeV in a large cyclotron. The protons then can be directed into any one of four treatment rooms. This arrangement minimizes "idle" time, because while one patient is being treated in one room the next two patients can be set up in adjoining treatment rooms. This sort of facility sets the scene for the future, that is, a large radiation-therapy facility with multiple treatment rooms in the context of a cancer center. (Courtesy of Drs. James Slater and John Archambeau, Loma Linda University, Loma Linda, California.)

## SUMMARY OF PERTINENT CONCLUSIONS

### Neutrons

- Neutrons are indirectly ionizing. In tissue they give up their energy to produce recoil protons,  $\alpha$ -particles, and heavier nuclear fragments.
- Biologic properties differ from x-rays in several regards: reduced OER, little or no repair of sublethal damage or potentially lethal damage, and less variation of sensitivity through the cell cycle.
- The rationale for the use of neutrons in radiotherapy has changed over the years. The earlier rationale was the reduced OER to overcome the problem of hypoxic cells. The revised rationale is based on a higher neutron RBE for slowly growing tumors.
- An advantage has been proved in clinical trials for neutrons in the treatment of salivary-gland and prostate tumors and soft-tissue sarcomas, but not for the majority of cancer sites tested.
- A new generation of hospital-based cyclotrons, generating neutrons by the  $p^+ \rightarrow Be$  reaction, are now in use.

### Boron Neutron-capture Therapy

- The principle is to deliver a drug containing boron that localizes only in tumors and then to treat with low-energy thermal neutrons that interact with boron to produce  $\alpha$ -particles.
- Boron is a suitable substance because it has a large cross-section for thermal neutrons and emits short-range densely ionizing  $\alpha$ -particles if bombarded by thermal neutrons. Its chemistry is such that it can be incorporated into a wide range of compounds.

- Many attempts have been made to synthesize boron-containing compounds that are selectively localized in tumors relative to normal tissues, with limited success. They fall into two categories:
  1. Low molecular weight agents that simulate chemical precursors needed for tumor proliferation
  2. High molecular weight agents such as monoclonal antibodies.
- Thermal neutrons are poorly penetrating in tissue, with a half-value layer of only 1.5 cm.
- Epithermal neutrons are somewhat more penetrating. They are degraded to thermal neutrons by collisions with hydrogen atoms in tissue. The peak dose is at 2 to 3 cm, and the high surface dose is avoided.
- Results of clinical trials of the efficacy of BNCT are tantalizing but not definitive.
- The concept of BNCT is very attractive, but formidable practical difficulties are involved in making it a practical treatment modality even for relatively shallow tumors.

### Protons

- Protons result in excellent physical dose distributions.
- Protons have biologic properties similar to x-rays.
- There is an established place for protons in the treatment of choroidal melanoma or tumors close to the spinal cord, in which a sharp cutoff of dose is important.
- Hospital-based high-energy cyclotrons with isocentric mounts are being used to treat a broader spectrum of cancer patients with protons. Their efficacy has yet to be proven.

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# 25

## Radiosensitizers and Bioreductive Drugs

THE HALOGENATED PYRIMIDINES  
RADIOSENSITIZING HYPOXIC CELLS  
HYPOXIC CYTOTOXINS

MARKERS OF HYPOXIC CELLS  
SUMMARY OF PERTINENT  
CONCLUSIONS

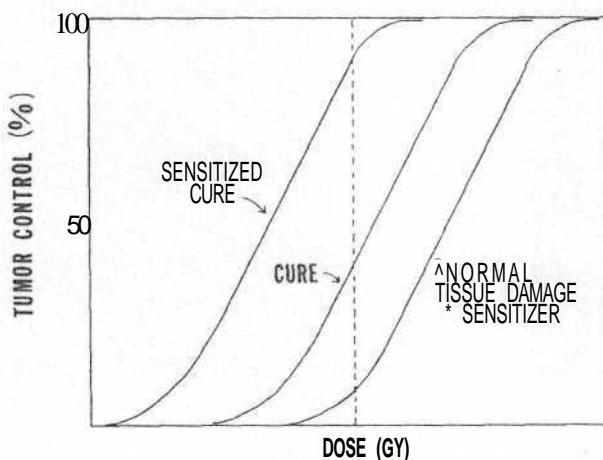
Radiosensitizers are chemical or pharmacologic agents that increase the lethal effects of radiation if administered in conjunction with it. Many compounds that modify the radiation response of mammalian cells have been discovered over the years, but most offer no practical gain in radiotherapy because they do not show a *differential effect* between tumors and normal tissues. There is no point in employing a drug that increases the sensitivity of tumor and normal cells to the same extent.

With this all-important criterion of a differential effect, only two types of sensitizers have found practical use in clinical radiotherapy:

1. The halogenated pyrimidines sensitize cells to a degree dependent on the amount of the analogue incorporated. In this case a differential effect is based on the premise that tumor cells cycle faster and, therefore, incorporate more of the drug than the surrounding normal tissues.
2. Hypoxic cell sensitizers increase the radiosensitivity of cells deficient in molecular oxygen but have no effect on normally aerated cells. In this case a differential effect is based on the premise that hypoxic cells occur only in tumors and not in normal tissues.

These two classes of sensitizers are discussed in turn. The basic strategy of all radiosensitizers is illustrated in **Figure 25.1**. The aim is to move

the tumor-control curve to lower doses by sensitizing tumor cells but not affecting the normal-tissue complication curve, or at least not altering it as much. The outcome would be to increase the tumor-control probability for a given level of normal-tissue complications.



**Figure 25.1.** The basic strategy of all radiosensitizers. The addition of the drug is expected to move the tumor-control curve to the left but not affect the normal-tissue complication curve, or at least not alter it as much. For halogenated pyrimidines this expectation is based on the premise that tumor cells are cycling more rapidly than cells of the dose-limiting normal tissues, so that they incorporate more sensitizer. For hypoxic cell radiosensitizers this expectation is based on the premise that hypoxic cells are present only in tumors, not in normal tissues, and these drugs preferentially sensitize hypoxic cells. (Based on an idea by Dr. Ged Adams.)

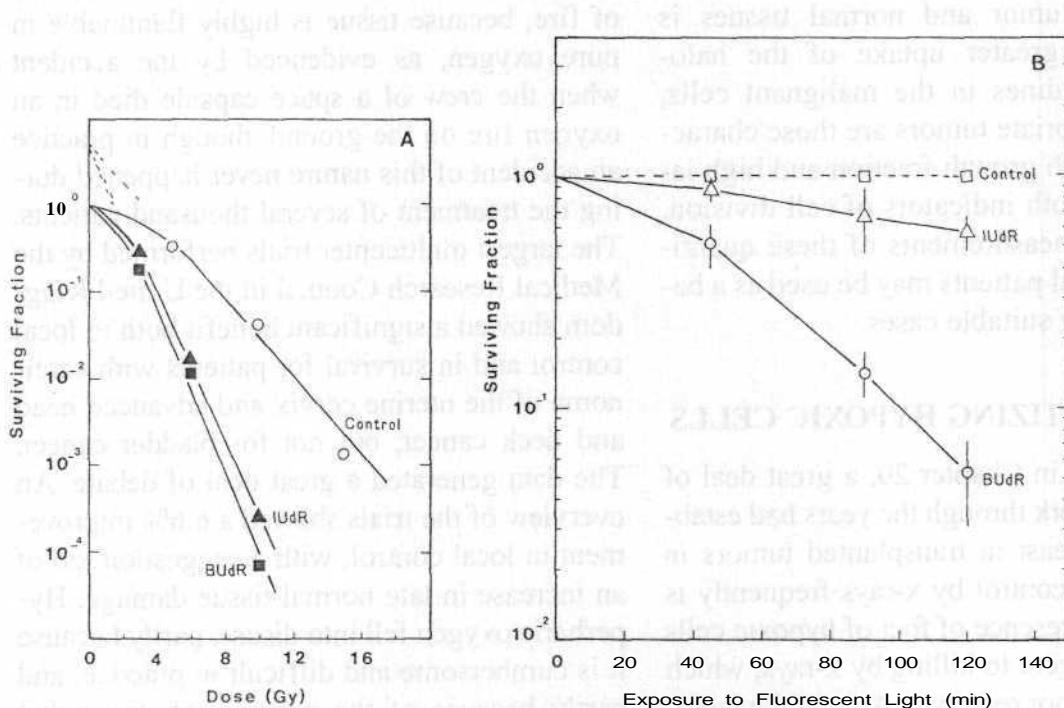
## THE HALOGENATED PYRIMIDINES

The combining size (the van der Waal radius) of an atom of chlorine, bromine, or iodine is very similar to that of the methyl group **CH<sub>3</sub>**. The halogenated pyrimidines, 5-iododeoxyuridine and 5-bromodeoxyuridine, consequently are very similar to the normal DNA precursor thymidine, having a halogen substituted in place of the methyl group.

The similarity is so close that they are incorporated into the DNA chain in place of thymidine. This substitution "weakens" the DNA chain, and consequently the cells are more susceptible to damage by  $\gamma$ -rays or ultraviolet light. These substances are effective as sensitizers only if they are made available to cells for several cell generations, so that an appreciable quantity of the analogue actually may be incorporated into the DNA. As the percentage of thymidine bases replaced increases, so does the extent of radiosensitization.

The effectiveness of the halogenated pyrimidines as sensitizers first was shown in bacteria, but a similar effect has been demonstrated amply in mammalian cells both *in vitro* and *in vivo*. Figure 25.2 shows the sensitization of hamster cells to x-rays and to fluorescent light by the incorporation of bromodeoxyuridine and iododeoxyuridine. Although there is little to choose between the bromine or the iodine analogues as far as sensitization to x-rays is concerned, bromodeoxyuridine is a much more efficient sensitizer for fluorescent light. This turns out to be an important difference in the clinical application of these drugs. One of the unpleasant side effects of bromodeoxyuridine in some patients is a rash, caused by phototoxicity from the interaction of light with the drug. This is much less of a problem with the iodine analogue, as might be predicted from Figure 25.2.

The use of halogenated pyrimidines as an adjunct to radiotherapy began in the 1970s. The



**Figure 25.2.** Survival curves for bromodeoxyuridine (BUdR)- and iododeoxyuridine (IUdR)-substituted cells exposed to x-rays (A) and fluorescent light (B). Both halogenated pyrimidines sensitize equally to x-rays, and there is little to choose between them on this count. Iododeoxyuridine, however, has little effect with fluorescent light; thus it is preferred clinically because it avoids the light-induced rash produced by bromodeoxyuridine. (From Mitchell J, Morstyn G, Russo A, et al.: Differing sensitivity to fluorescent light in Chinese hamster cells containing equally incorporated quantities of BUdR versus IUdR. *Int J Radiat Oncol Biol Phys* 10:1447-1451, 1984, with permission.)

rationale was that tumor cells may be cycling more rapidly than the normal cells in surrounding tissues, so that more drug could be replaced in the tumor cell DNA, resulting in "selective" radiosensitization. It was perhaps unfortunate that head-and-neck tumors were among those treated in this early study at Stanford University, because they are surrounded by actively proliferating normal tissues. The choice of tumors in the head and neck was determined partly by the need to deliver the analogue by an intraarterial infusion into the main vessel supplying the neoplasm to be treated, because the liver tends to dehalogenate the circulating drug. Tumor responses were reported to be good, but **normal-tissue** damage was unacceptable.

Consequently, these sensitizers were not used for a number of years, until several centers began an evaluation **in** more suitable tumor sites, in which the proximity of actively proliferating normal tissues is not such a problem. Among the tumors evaluated were high-grade glioblastomas and large unresectable sarcomas.

Because the all-important differential effect between tumor and normal tissues is based on the greater uptake of the halogenated pyrimidines in the malignant cells, the most appropriate tumors are those characterized by a high growth fraction and high labeling index, both indicators of cell division. In the future, measurements of these quantities **in** individual patients may be used as a basis for choosing suitable cases.

### RADIOSENSITIZING HYPOXIC CELLS

As described **in** Chapter 20, a great deal of experimental work through the years had established that, at least in transplanted tumors in animals, tumor control by x-rays frequently is limited by the presence of foci of hypoxic cells that are intransigent to killing by x-rays, which may result **in** tumor regrowth. Among the methods suggested to overcome this problem are treatment in hyperbaric oxygen chambers and the introduction of high linear energy transfer radiations, such as neutrons and heavy ions. Chemical sensitizers address the same problem. High linear energy transfer radiations are dis-

cussed in Chapter 7. This chapter addresses hyperbaric oxygen, chemical radiosensitizers, and the latest approach, namely, hypoxic cytotoxins.

### Hyperbaric Oxygen

Following the identification of hypoxia as a possible source of tumor resistance, a major effort was made to solve the problem by the use of hyperbaric oxygen. Patients were sealed in chambers filled with pure oxygen raised to a pressure of three atmospheres. Churchill Davidson at St. Thomas' Hospital in London pioneered this work, but it was taken up by researchers on both sides of the Atlantic. The clinical **trials** that were performed involved small numbers of patients and were difficult to interpret because unconventional fractionation schemes were used; that is, a few large fractions were used because of the time and effort involved in the technical procedures. Patient compliance was also a problem because of the feeling of claustrophobia from being sealed in a narrow tube. There was also the serious risk of fire, because tissue is highly flammable in pure oxygen, as evidenced by the accident when the crew of a space capsule died in an oxygen fire on the ground, though in practice an accident of this nature never happened during the treatment of several thousand patients. The largest multicenter trials performed by the Medical Research Council in the United Kingdom showed a significant benefit both in local control and in survival for patients with carcinoma of the uterine **cervix** and advanced head and neck cancer, but not for bladder cancer. The data generated a great deal of debate. An overview of the trials showed a 6.6% improvement in local control, with a suggestion too of an increase in late **normal-tissue** damage. Hyperbaric oxygen fell into disuse, partly because it is cumbersome and difficult in practice, and partly because of the promise of drugs that would achieve the same end by simpler means.

The notion of improving tumor oxygenation by breathing 100% oxygen rather than air has been revived in recent years by experiments involving carbogen. If pure oxygen is breathed it tends to lead to vasoconstriction, a

closing down of some blood vessels, which of course defeats the object of the exercise. This is avoided if 5% carbon dioxide is added to the oxygen, a mixture called *carbogen*. Breathing carbogen at atmospheric pressure, then, is a relatively simple attempt to overcome chronic hypoxia, that is, diffusion-limited hypoxia. The use of carbogen in combination with nicotinamide is described subsequently in this chapter.

### Improving the Oxygen Supply to Tumors

A group at the Princess Margaret Hospital in Toronto showed convincingly that a blood transfusion prior to radiotherapy led to a significant improvement of local tumor control probability in patients with carcinoma of the uterine cervix. A number of other studies have shown that hemoglobin levels can influence the success of radiation therapy.

Tumor oxygenation also can be improved by the use of artificial blood substances such as perfluorocarbons. Smoking also can influence tumor oxygenation, and it is clearly advisable for patients to give up smoking, at least during radiotherapy.

### Hypoxic Cell Radiosensitizers

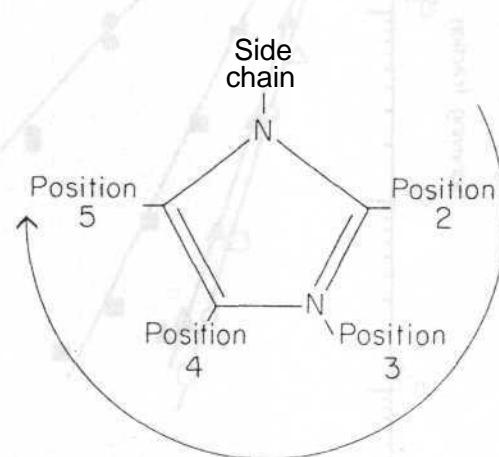
Spurred largely by the efforts of radiation chemists (most notably Adams), a search was under way in the early 1960s for compounds that mimic oxygen in their ability to sensitize biologic materials to the effects of x-rays. Instead of trying to "force" oxygen into tissues by the use of high-pressure tanks, the emphasis of this approach shifted to oxygen substitutes that diffuse into poorly vascularized areas of tumors and achieve the desired effect by chemical means. The vital difference between these drugs and oxygen, on which their success depends, is that the sensitizers are not rapidly metabolized by the cells in the tumor through which they diffuse. Because of this, they can penetrate further than oxygen and reach all of the hypoxic cells in the tumor, including those most remote from a blood supply. In the early 1960s, many simple chemical compounds were found to have the ability to sensitize hypoxic

microorganisms. These studies were guided by the hypothesis, now known to be correct, that sensitizing efficiency is related directly to the electron affinity of the compounds.

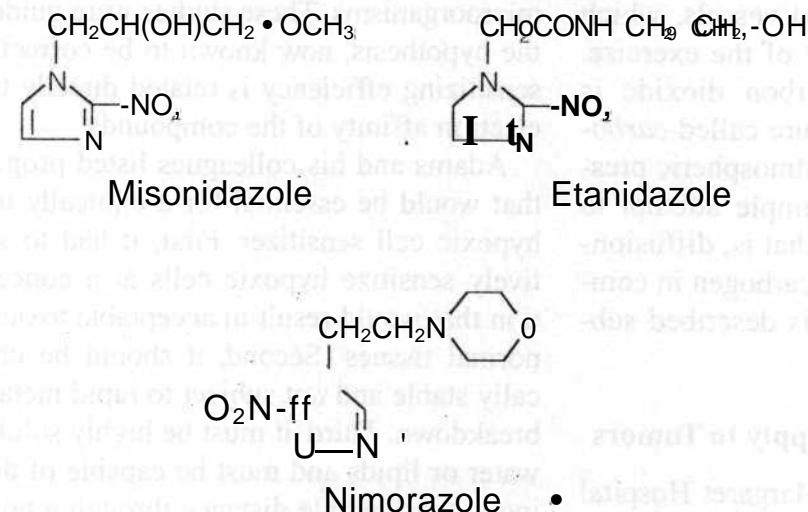
Adams and his colleagues listed properties that would be essential for a clinically useful hypoxic cell sensitizer. First, it had to selectively sensitize hypoxic cells at a concentration that would result in acceptable toxicity to normal tissues. Second, it should be chemically stable and not subject to rapid metabolic breakdown. Third, it must be highly soluble in water or lipids and must be capable of diffusing a considerable distance through a nonvascularized cell mass to reach the hypoxic cells, which in a tumor may be located as far as 200  $\mu\text{m}$  from the nearest capillary. Fourth, it should be effective at the relatively low daily doses of a few grays (a few hundred rads) used in conventional fractionated radiotherapy. The first candidate compound that appeared to satisfy these criteria was misonidazole.

### Misonidazole

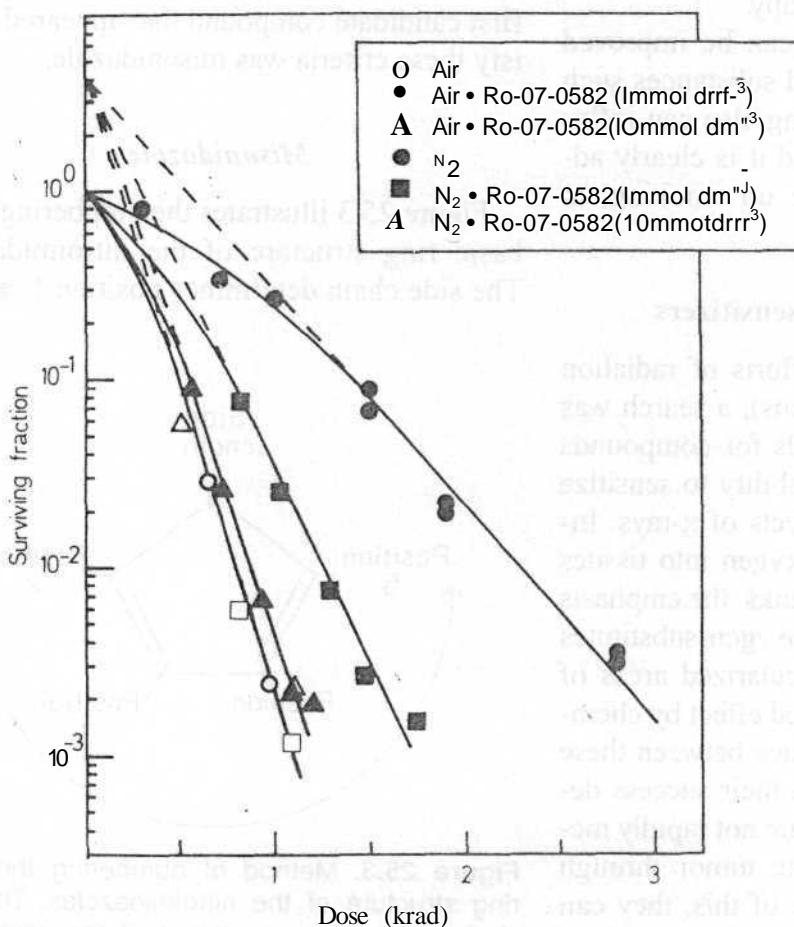
Figure 25.3 illustrates the numbering of the basic ring structure of the nitroimidazoles. The side chain determines position 1, and the



**Figure 25.3.** Method of numbering the basic ring structure of the nitroimidazoles. The side chain determines position 1. If the nitro group ( $\text{NO}_2$ ) is in the second position, the drug is a 2-nitroimidazole. If the  $\text{NO}_2$  group is in the fifth position, the drug is a 5-nitroimidazole. In general, 2-nitroimidazoles are more efficient radiosensitizers of hypoxic cells.



**Figure 25.4.** The structure of misonidazole, etanidazole, and nimorazole, the three compounds used most widely in clinical trials. Misonidazole and etanidazole are 2-nitroimidazoles; nimorazole is a 5-nitroimidazole. Misonidazole and etanidazole are equally active as radiosensitizers, but etanidazole is less neurotoxic because it has a shorter half-life and is hydrophilic. Nimorazole is less active but very much less toxic than either misonidazole or etanidazole, so that larger doses are tolerable.



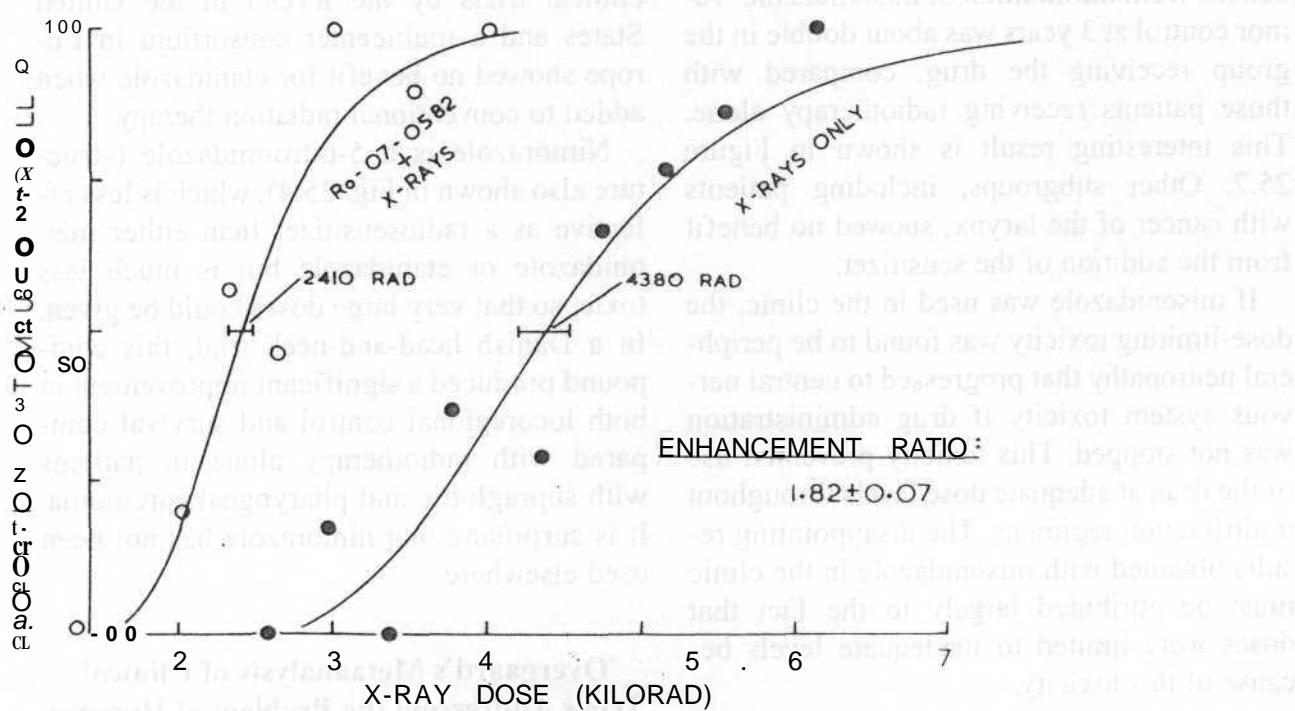
**Figure 25.5.** Survival data for aerated and hypoxic Chinese hamster cells x-irradiated in the presence of various concentrations of misonidazole (Ro-07-0582). At a concentration of 10 mM of this drug the radiosensitivity of hypoxic cells approaches that of aerated cells. The response of aerated cells is not affected by the drug at all. (From Adams GE, Flockhart IR, Smithen CE, Stratford IJ, Wardman R, Watts ME: Radiat Res 67:9-20, 1976, with permission.)

position of the nitro group ( $\text{NO}_2$ ) leads to the classification of the drug as a 2-nitroimidazole, 4-nitroimidazole, or so on. In general, 2-nitroimidazoles have a higher electron affinity than 5-nitroimidazoles, the class that includes metronidazole which was briefly tried as a radiosensitizer. Misonidazole is a 2-nitroimidazole; its structure is shown in Figure 25.4.

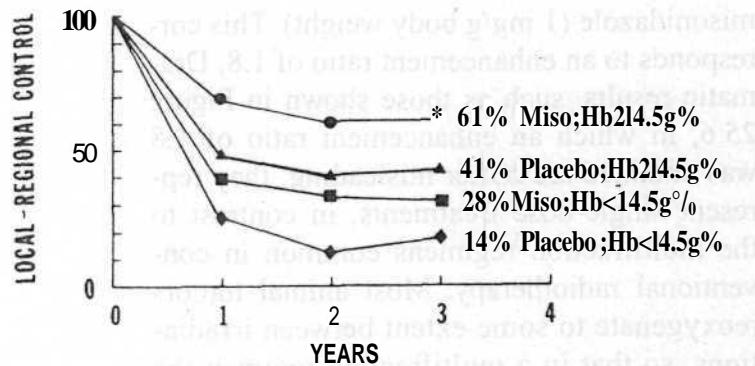
Misonidazole produces appreciable sensitization with cells in culture (Fig. 25.5). Hypoxic cells in the presence of 10 mM of misonidazole have a radiosensitivity approaching that of aerated cells. Misonidazole also has a dramatic effect on tumors in experimental animals. This is illustrated in Figure 25.6, which shows the proportion of mouse mammary tumors controlled as a function of x-ray dose delivered in a single fraction. If x-rays are used alone, the dose required to control half of the tumors is 43.8 Gy (4,380 rad). This falls to 24.1 Gy (2,410 rad) if the radiation is delivered 30 minutes after the administration of

misonidazole (1 mg/g body weight). This corresponds to an enhancement ratio of 1.8. Dramatic results, such as those shown in Figure 25.6, in which an enhancement ratio of 1.8 was obtained are rather misleading; they represent single-dose treatments, in contrast to the multifraction regimens common in conventional radiotherapy. Most animal tumors reoxygenate to some extent between irradiations, so that in a multifraction regimen the enhancement ratio for a hypoxic sensitizer is usually much less than for a single-dose treatment.

After encouraging results in laboratory studies, misonidazole was introduced into a large number of clinical trials, involving many different types of human tumors, in Europe and the United States. In general, the results have been disappointing. Of the 20 or so randomized prospective controlled clinical trials performed in the United States by the Radiation Therapy Oncology Group (RTOG), none yielded a statistically significant advantage



**Figure 25.6.** Proportion of mouse mammary tumors controlled at 150 days as a function of x-ray dose for a single treatment. The *right curve* represents x-rays only; the *left curve* refers to x-rays delivered after the administration of 1 mg/g body weight of misonidazole (Ro-07-0582). The enhancement ratio is the ratio of x-ray doses in the absence or presence of the drug that results in the control of 50% of the tumors; it has a value of 1.8. (From Sheldon PW, Foster JL, Fowler JF: Br J Cancer 30:560-565, 1974, with permission.)



**Figure 25.7.** Some results from the Danish head-and-neck trial of misonidazole. Misonidazole produced a significant improvement of tumor control by radiotherapy only for males with tumors of the pharynx and depended on hemoglobin status. (Data from Dr. Jens Overgaard.)

for misonidazole, although a number indicated a slight benefit. The only trial that shows a clear advantage for misonidazole was the head-and-neck trial performed in Denmark, the largest single trial performed with the sensitizer. If patients of all categories are compared in this trial, the addition of misonidazole to the radiotherapy schedule conferred no significant advantage. If the patients are categorized into a number of subgroups, however, males with high hemoglobin levels and cancer of the pharynx showed a great benefit from the addition of misonidazole. Tumor control at 3 years was about double in the group receiving the drug, compared with those patients receiving radiotherapy alone. This interesting result is shown in Figure 25.7. Other subgroups, including patients with cancer of the larynx, showed no benefit from the addition of the sensitizer.

If misonidazole was used in the clinic, the dose-limiting toxicity was found to be peripheral neuropathy that progressed to central nervous system toxicity if drug administration was not stopped. This toxicity prevented use of the drug at adequate dose levels throughout multifraction regimens. The disappointing results obtained with misonidazole in the clinic must be attributed largely to the fact that doses were limited to inadequate levels because of this toxicity.

#### *Etanidazole and Nimorazole*

Spurred by the promise of misonidazole in the laboratory compared with its failure in the

clinic, efforts were made to find a better drug. The compound chosen for the next series of clinical trials in the United States was etanidazole, a 2-nitroimidazole, the structure of which also is shown in Figure 25.4. This compound equals misonidazole as a sensitizer but is less toxic, so that doses could be increased by a factor of 3. The lower neurotoxicity is a function of a shorter half-life *in vivo* plus a lower partition coefficient, so that it penetrates poorly into nerve tissue and does not cross the blood-brain barrier. Controlled clinical trials by the RTOG in the United States and a multicenter consortium in Europe showed no benefit for etanidazole when added to conventional radiation therapy.

Nimorazole is a 5-nitroimidazole (structure also shown in Fig. 25.4), which is less effective as a radiosensitizer than either misonidazole or etanidazole but is much less toxic, so that very large doses could be given. In a Danish head-and-neck trial, this compound produced a significant improvement in both locoregional control and survival compared with radiotherapy alone in patients with supraglottic and pharyngeal carcinoma. It is surprising that nimorazole has not been used elsewhere.

#### **Overgaard's Metaanalysis of Clinical Trials Addressing the Problem of Hypoxia**

For over three decades an enormous effort has been expended in an attempt to overcome the perceived problem of hypoxia. Dozens of clinical trials have been performed, most of

which have been inconclusive or showed results with borderline significance. Overgaard and colleagues performed a metaanalysis, in which the results of all of the trials were combined and analyzed together. They identified 10,602 patients treated in 82 randomized clinical trials, involving hyperbaric oxygen, chemical sensitizers, carbogen breathing, or blood transfusions. Tumor sites included the bladder, uterine cervix, central nervous system, head and neck, and lung.

Overall, local tumor control was improved by 4.6%, **survival** by 2.8%, and the complication rate increased by only 0.6%, which was not statistically significant. The largest number of trials involved head-and-neck tumors, which also showed the greatest benefit. It also was concluded that the problem of hypoxia may be marginal in most adenocarcinomas and most important in squamous cell carcinomas.

The development of nitroimidazoles is illustrated by the following:

**Metronidazole**  
**I**

Misonidazole: more active, toxic; benefit in subgroups

**i**

Etanidazole: less toxic, no benefit

**I**

Nimorazole: less active, much less toxic; benefit in head-and-neck cancer

### Nicotinamide and Carbogen Breathing

Hypoxic cell radiosensitizers, such as the nitroimidazoles, were designed primarily to overcome chronic hypoxia, that is, diffusion-limited hypoxia resulting from the inability of oxygen to diffuse further than about 100  $\mu$ m through respiring tissue. As explained in the chapter on oxygen (Chapter 6), however, there is also another form of hypoxia known as *acute hypoxia*, that is, local regions of hypoxia caused by the intermittent closing down of blood vessels. Nicotinamide, a vitamin B3 analogue, prevents these transient fluctuations in tumor blood flow that lead to the development of acute hypoxia, at least in mouse tumors.

A combination of nicotinamide to overcome acute hypoxia and carbogen breathing to overcome chronic hypoxia is the basis of the ARCON trials that are underway in a number of European centers. The trials are also accelerated and hyperfractionated, to avoid tumor proliferation and damage to late-responding normal tissues. A summary of the ARCON treatment follows:

Accelerated to overcome proliferation

Hyperfractionated to spare late-responding normal tissues

Carbogen breathing to overcome chronic hypoxia

Nicotinamide to overcome acute hypoxia

### HYPOXIC CYTOTOXINS

An alternative approach to designing drugs that preferentially *radiosensitize* hypoxic cells is to develop drugs that selectively *kill* hypoxic cells. It was pointed out at an early stage that the greater reductive environment of tumors might be exploited by developing drugs that are reduced preferentially to cytotoxic species in the hypoxic regions of tumors. Three classes of agents in this category are known:

1. The quinone antibiotics
2. Nitroaromatic compounds
3. The benzotriazine di-N-oxides.

Mitomycin C is an example of the first class and has been used as a chemotherapy agent, active against hypoxic cells, for many years. The aerated-hypoxic differential, however, is relatively small for these compounds. Examples of the second class of compounds include dual-function agents developed by Adams and his group at the Medical Research Council radiobiology unit in England. Normal-tissue toxicity prevented the trial of these compounds in the clinic. The lead compound of the third class is tirapazamine and shows highly selective toxicity towards hypoxic cells both *in vitro* and *in vivo*.

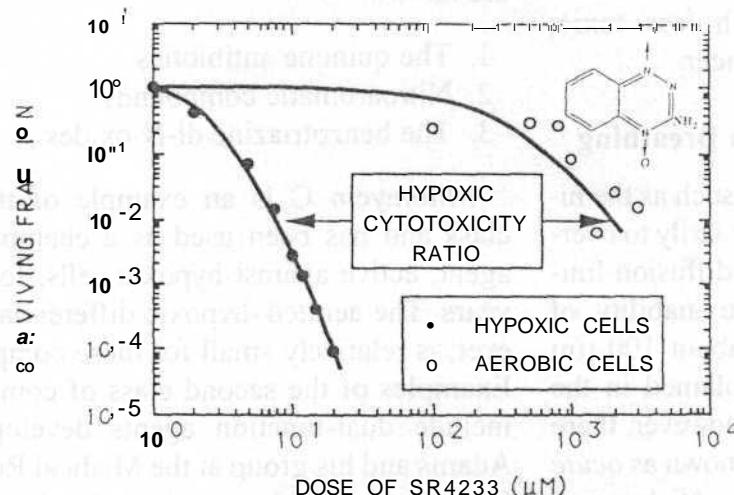
### Tirapazamine

Figure 25.8 shows survival curves for Chinese hamster cells treated with graded concentrations of tirapazamine. Note the hypoxic/oxic toxicity ratio of about 100. This compound is believed to be activated by the enzyme cytochrome P450. The hypoxic/oxic toxicity ratio is not as large (about 20) in cell lines of human origin, presumably reflecting a different spectrum, or different levels, of enzymes.

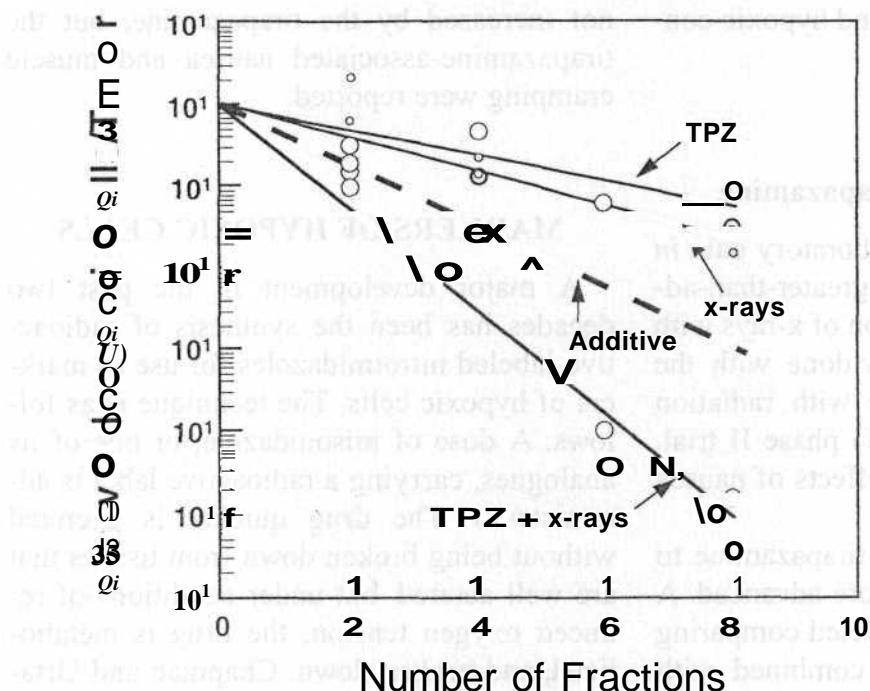
Figure 25.9 shows the results of an experiment in which a transplanted mouse carcinoma was treated with x-rays alone, tirapazamine alone, or a combination of the two, with the drug injected 30 min prior to each irradiation. The irradiation schedule consisted of 2.5-Gy fractions given twice daily. Following treatment, the tumors were removed and the cells assayed for clonogenic survival. The effect of x-rays plus tirapazamine is evidently much greater than additive, as would be expected from independent cell killing of the two agents. The effect was even more dramatic when the study was conducted entirely *in vivo*, scoring regrowth delay (Fig. 25.10). Tumors were treated with x-rays alone, tirapazamine

alone, or a combination of both agents. The radiation schedule consisted of eight 2.5-Gy (250-rad) fractions designed to mimic as far as possible a clinical radiation therapy protocol. The combination of drug and radiation is highly effective, with the time sequence of drug before radiation slightly more effective than the reverse. In parallel experiments using the same x-ray and drug protocols, skin reactions were scored and no radiosensitization or additive cytotoxicity was observed by the addition of the tirapazamine to the radiation treatments. This substantiates the tumor selectivity of the radiation enhancement.

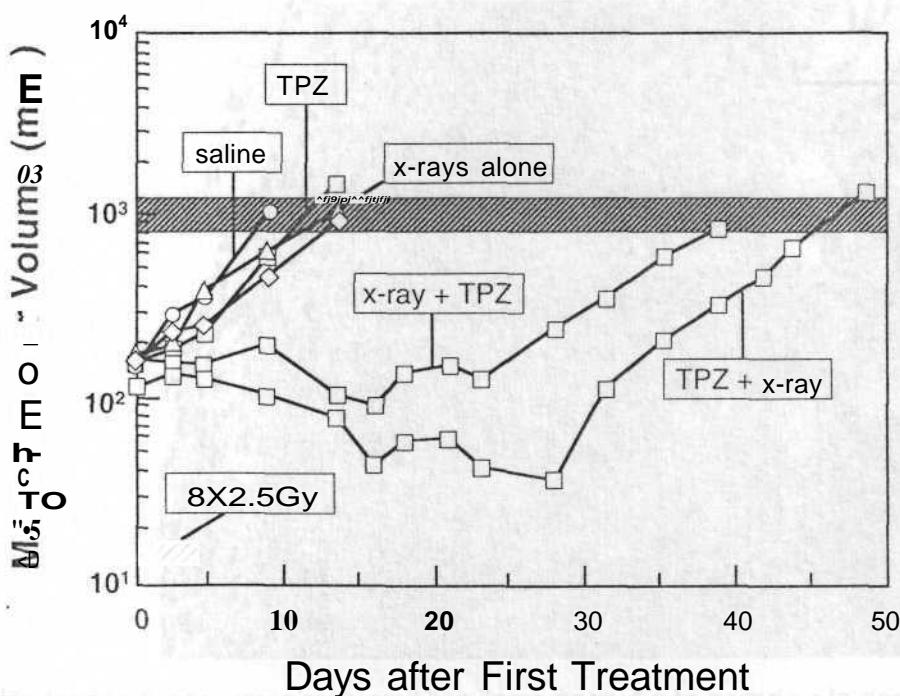
Similar results were obtained in four different mouse tumors that differed significantly in their hypoxic fractions. The observed interaction between x-rays and tirapazamine results largely from the selective hypoxic toxicity of the drug. This does not totally explain the observations, however. It appears that tirapazamine can act as an aerobic radiosensitizer of cells exposed to the drug under hypoxic conditions before or after the aerobic irradiation. This latter mechanism would be important for intermittent or acute hypoxia, that is, where a given region of a tu-



**Figure 25.8.** Dose-response curves for Chinese hamster cells exposed for 1.5 hours to graded concentrations of SR 4233 (tirapazamine) under aerated and hypoxic conditions. Cells deficient in oxygen are killed preferentially. The hypoxic cytotoxicity ratio (defined as the ratio of drug concentrations under aerated and hypoxic conditions required to produce the same cell survival) is variable between different cell lines. For Chinese hamster cells shown, the ratio is about 100; for cells of human origin the ratio is somewhat smaller, closer to 20. Tirapazamine is an organic nitroxide synthesized by Stanford Research International. Its structure is shown in the inset. (Courtesy of Dr. J. Martin Brown.)



**Figure 25.9.** Response of SCCVII mouse carcinomas to tirapazamine (TPZ) alone, radiation alone, or a combination of both. The radiation or drug treatment was given every 12 hours for up to eight fractions and the mice sacrificed for clonogenic cell survival 12 hours after the last dose. The additive line is the survival expected for independent cell killing by x-rays and tirapazamine, assuming a homogeneous tumor cell population. The actual killing observed is clearly greater than this. (Adapted from Brown JM, Lemmon MJ: Potentiation by the hypoxic cytotoxin SR4233 of cell killing produced by fractionated irradiation of mouse tumors. *Cancer Res* 50:7745-7749, 1990, with permission)



**Figure 25.10.** Tumor volume as a function of time after various treatments of a SCCVII transplantable mouse carcinoma. Tirapazamine or radiation alone had little effect. The combination of tirapazamine and x-rays caused significant growth delay, with radiation following the drug causing a slightly greater effect. (Adapted from Brown JM, Lemmon MJ: Tumor hypoxia can be exploited to preferentially sensitize tumors to fractionated irradiation. *Int J Radiat Oncol Biol Phys* 20:457-461, 1991, with permission.)

mor cycles between aerated and hypoxic conditions.

### Clinical Trials with Tirapazamine

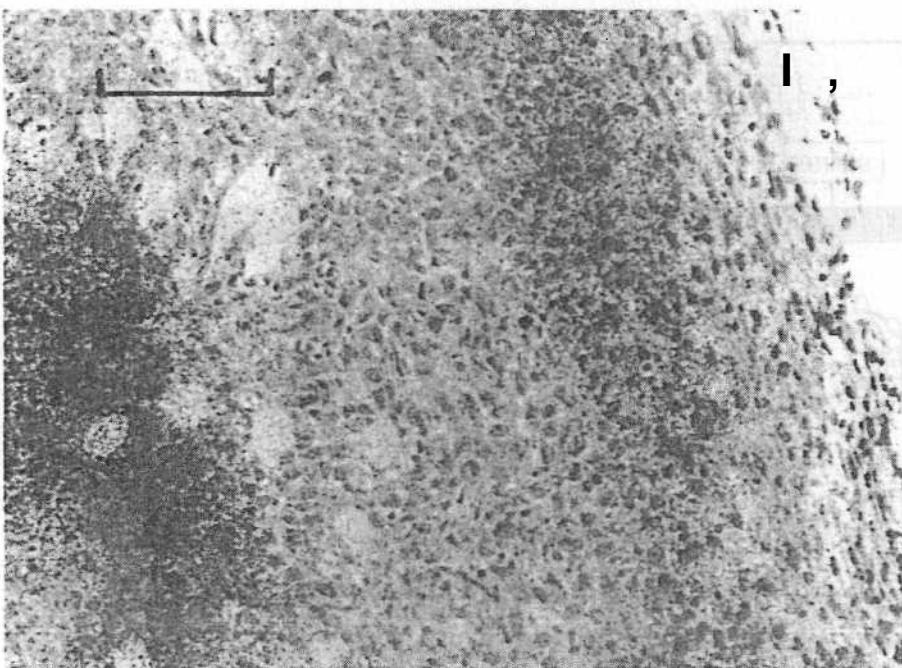
In spite of the extensive laboratory data *in vitro* and *in vivo*, showing the greater-than-additive effect of the combination of x-rays with tirapazamine, little has been done with the clinical use of tirapazamine with radiation therapy, except for one RTOG phase II trial. The reason may be the side effects of nausea and severe muscle cramping.

The situation with adding tirapazamine to chemotherapy protocols is more advanced. A phase III trial has been completed comparing cisplatin alone, or cisplatin combined with tirapazamine for advanced (stage IIB and IV) non-small cell lung cancer. There was a doubling of response rates and an increased survival rate in patients receiving the drug combination. The systemic cisplatin toxicity was

not increased by the tirapazamine, but the tirapazamine-associated nausea and muscle cramping were reported.

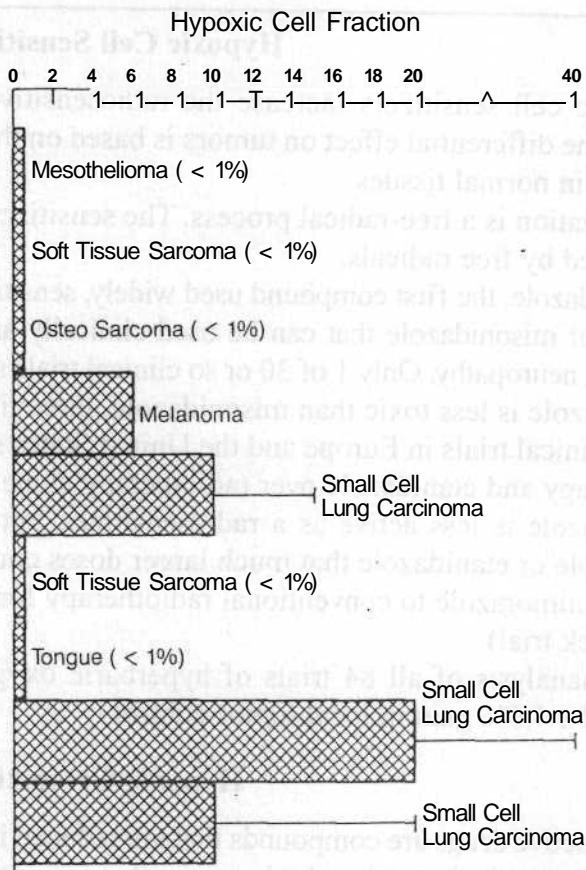
### MARKERS OF HYPOXIC CELLS

A major development in the past two decades has been the synthesis of radioactive-labeled nitroimidazoles for use as markers of hypoxic cells. The technique is as follows: A dose of misonidazole, or one of its analogues, carrying a radioactive label is administered. The drug quickly is excreted without being broken down from tissues that are well aerated, but under conditions of reduced oxygen tension, the drug is metabolized, and broken down. Chapman and Urtasun in Edmonton, Canada, performed a study in which patients were given a nitroimidazole labeled with tritiated thymidine 22 hours before a tumor was removed surgically. Figure 25.11 is a section of a melanoma showing the



**Figure 25.11.** Autoradiograph of a section of a human small cell lung carcinoma from a patient who received radioactive-labeled-misonidazole the previous day. There are areas of intense labeling (many grains in the emulsion), suggesting the presence of hypoxic regions in the tumor. In areas deficient in oxygen the misonidazole undergoes anaerobic metabolism and is broken down, and the radioactive label is deposited. (Courtesy of Drs. J. D. Chapman and R. Urtasun.)

density of grains over a region of the tumor, indicating the presence of hypoxic cells. The interesting result of the study is that only four of nine patients had tumors with a significant proportion of hypoxic cells (Fig. 25.12). In this small group of patients, biased inasmuch as only accessible tumors could be studied, only melanoma and small-cell lung cancer appeared to contain a proportion of hypoxic cells that would prejudice the outcome of radiotherapy. Until recently it was possible only to use  $\gamma$ -emitting isotopes as labels, which can be detected only by autoradiography when the tumor is removed and sectioned. More recently, Chapman has successfully labeled a nitroimidazole by attaching a positron-emitting radionuclide (iodine-123) using a sugar molecule. The presence of hypoxia then may be detected by single photon emission computed tomography imaging, which of course is noninvasive. This exciting development opens up the possibility of screening patients prospectively to select those in whom hypoxia is a problem for inclusion in protocols involving hypoxic cell sensitizers or bioreductive drugs. This topic is discussed further in the chapter on predictive assays (Chapter 23).



**Figure 25.12.** Summary of the labeling levels found in the first eight patients to be administered radioactive-labeled misonidazole. Only four of the eight patients show levels of labeling commensurate with the presence of hypoxic cells. (Courtesy of Drs. J. D. Chapman and R. Urtasun.)

## SUMMARY OF PERTINENT CONCLUSIONS

### Nonhypoxic Sensitizers

- If the methyl group in thymidine is replaced by a halogen and incorporated into DNA, it results in radiosensitization.
- The halogenated pyrimidine must be incorporated into DNA for sensitization to occur. Consequently, cells must be grown in the presence of the analogue for several cell cycles. The extent of radiosensitization increases with the amount incorporated.
- For a sensitizer to be useful there must be a differential effect between tumors and normal tissues. The halogenated pyrimidines require the tumor to be cycling faster than the **dose-limiting** normal tissues.
- Iododeoxyuridine is preferred to bromodeoxyuridine because it is an equally effective radiosensitizer but a less effective photosensitizer. Rash therefore is reduced in patients.
- Gliomas may be suitable for clinical studies because they are rapidly growing and surrounded by slowly growing or nongrowing normal tissue.

### Hypoxic Cell Sensitizers

- Hypoxic cell sensitizers increase the radiosensitivity of hypoxic cells but not aerated cells. The differential effect on tumors is based on the presence of hypoxic cells in tumors but not in normal tissues.
- Sensitization is a free-radical process. The sensitizer mimics oxygen by "fixing" damage produced by free radicals.
- Misonidazole, the first compound used widely, sensitizes cells in culture and animal tumors.
- Doses of misonidazole that can be used clinically are limited to suboptimal levels by peripheral neuropathy. Only 1 of 30 or so clinical trials showed an advantage for misonidazole.
- Etanidazole is less toxic than misonidazole, three times larger doses are tolerated—however, clinical trials in Europe and the United States showed no advantage of combined radiotherapy and etanidazole over radiotherapy alone.
- Nimorazole is less active as a radiosensitizer, but so much less toxic than either misonidazole or etanidazole that much larger doses could be given. A benefit was shown for adding nimorazole to conventional radiotherapy for head-and-neck cancer (Danish head-and-neck trial).
- A metaanalysis of all 84 trials of hyperbaric oxygen and hypoxic cell radiosensitizers showed a 4.6% gain in local tumor control.

### Hypoxic Cytotoxins

- Bioreductive drugs are compounds that are reduced intracellularly to form cytotoxic agents.
- Bioreduction is favored under hypoxia; this is a rationale for selectivity **in** solid tumors.
- Mitomycin C is active in a wide range of tumors. The hypoxic/oxic cytotoxicity ratio is quite small.
- Tirapazamine is an organic nitroxide. It shows a large hypoxic/oxic toxicity ratio. The compound is active in many animal tumors and has undergone several clinical trials in conjunction with radiation or chemotherapy agents.
- Tirapazamine was designed to be used as an adjunct to irradiation, with the radiation killing the aerobic cells and the drug targeted to kill the hypoxic cells. It has found more use as an adjunct to chemotherapy agents.

### Markers of Hypoxic Cells

- Nitroimidazoles labeled with a radionuclide can be used as markers of hypoxic cells. The drug quickly is excreted from aerobic tissues without breaking down. In areas of hypoxia, the drug undergoes bioreduction and the radionuclide is deposited.
- Nitroimidazoles labeled with a p-emitter have been available for some time. By autoradiography on sections of a tumor surgically removed about a day after administration of a labeled drug, hypoxic areas can be identified. Only a minority of human tumors (notably melanomas and small cell lung carcinomas) showed an appreciable proportion of hypoxic cells.
- Nitroimidazoles now can be labeled with iodine-123. Hypoxic regions of a tumor can be visualized by single-photon emission computed tomographic scanning. This is a noninvasive procedure that can be used as a predictive assay in individual patients. In the few patients tested to date, about 40% show the presence of hypoxic areas.
- The availability of methods to detect significant areas of hypoxia will allow selection of patients who may benefit from methods of overcoming hypoxia (*e.g.*, radiosensitizers, hypoxic cytotoxins, neutrons).

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## Gene Therapy

### SUICIDE-GENE THERAPY

#### CYTOTOXIC VIRUS TARGETED TO p53-

#### DEFICIENT CELLS

#### MOLECULAR IMMUNOLOGY (*i.e.*, CANCER VACCINES)

#### TUMOR-SUPPRESSOR GENE THERAPY

### RADIATION-INDUCIBLE GENE LINKED TO

#### A CYTOTOXIC AGENT

### TARGETING GENE THERAPY

### CONCLUSION

### SUMMARY OF PERTINENT

### CONCLUSIONS

"Gene therapy" is a catch-all term that covers a number of quite different new approaches to cancer treatment. Common to them all is the need for a means to introduce a gene into the cells of a tumor. At the present time, viral vectors are the most obvious choice and the most commonly used. The various types of viruses have different pros and cons, as summarized here:

**Retrovirus:** Convenient, but infects only dividing cells

**Adenovirus:** Infects both dividing and quiescent cells, but invokes an immune response, so that multiple applications are difficult

**Herpesvirus:** Attractive because it is bigger, allows more to be packaged into it—but more pathogenic and difficult to control

**Retroviruses** are convenient to work with but infect only dividing cells. This is a severe limitation. Adenoviruses infect both dividing and quiescent cells, but the downside to their use is that they evoke an immune response, which can make their repeated use difficult.

In most instances, the virus is used simply to deliver a gene of interest to the cells, in which case, in the interest of safety, the virus is engineered so that it is not replication-com-

petent. In a few instances the virus is designed to be cytotoxic, in which case some means is sought to selectively limit its activity to tumor cells only.

The **herpesvirus** is attractive because it is a much bigger virus, which allows more to be packaged into it, but it is also potentially more pathogenic and difficult to control.

There are at least five quite different approaches to gene therapy. These are described very briefly, in turn:

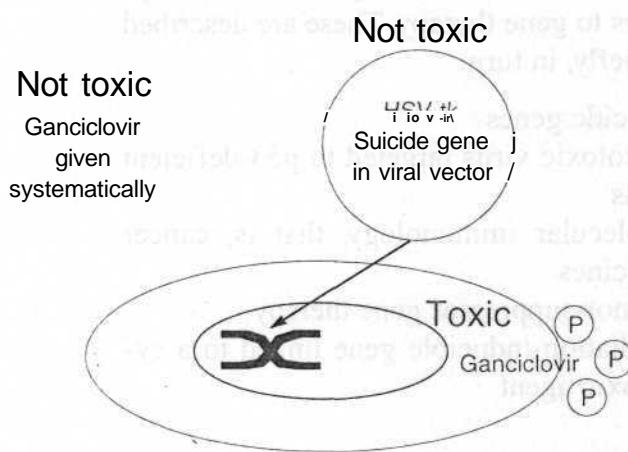
1. Suicide genes
2. Cytotoxic virus targeted to p53-deficient cells
3. Molecular immunology, that is, cancer vaccines
4. Tumor-suppressor gene therapy
5. Radiation-inducible gene linked to a cytotoxic agent

### SUICIDE-GENE THERAPY

Suicide-gene therapy is based on the strategy of transducing a gene into cells that converts an inert prodrug into a toxic agent. It is axiomatic that, alone, both the gene product and the prodrug are essentially non-toxic. Two such systems **have** been investi-

gated widely in experimental systems and in some clinical trials. One is described, briefly, as an example, namely the combination of the herpes simplex virus thymidine kinase gene (*RSV-tk*) packaged into an adenovirus plus ganciclovir. The virus containing the gene is injected into the tumor; the ganciclovir is administered systemically. HSV-A: phosphorylates ganciclovir, converting it into a nucleoside analogue that inhibits DNA synthesis, leading to cell death. The idea is illustrated in Figure 26.1. More cells are killed than are transduced initially, that is, there is a substantial "bystander" effect, resulting from several causes: (1) Gap junctions between cells transport the toxic agent to cells not themselves transduced; (2) as cells die and are lysed, the toxic agent is released and spreads to other cells; and (3) an induced immune response kills more tumor cells, even some remote from the injection site.

More than 30 clinical trials have been performed for human malignancies as diverse as brain tumors, mesotheliomas, liver metastases, and peritoneal metastases. Al-



**Figure 26.1.** The principle of suicide-gene therapy. The thymidine kinase gene from the herpes simplex virus (HSV-f/c), contained in a viral vector (adenovirus, so that dividing and nondividing cells can be infected), is injected into the tumor. Ganciclovir is administered systemically. This is a prodrug that is in itself nontoxic. In cells containing the thymidine kinase gene, the prodrug is activated to a toxic agent.

though local treatments are tolerated well, attempts at systemic delivery of the HSV-A: to target metastatic disease is limited by liver damage caused by hepatotropism of the adenovirus

Tumor growth reductions by *HSV-tk* plus ganciclovir are sometimes impressive, but cure rates are low, pointing to the need to use this new form of therapy as an adjuvant to radiation or chemotherapy. In fact, the combined therapy may have a greater-than-additive effect, because *HSV-tk* plus ganciclovir can radiosensitize cells in a manner similar to that shown for other nucleoside analogues.

#### CYTOTOXIC VIRUS TARGETED TO p53-DEFICIENT CELLS

A novel strategy to differentially treat tumors (both primary and metastatic) that are characterized by mutant p53 involves the use of an adenovirus that replicates only within p53 mutant cells, killing them through cell lysis. The basic biology involved in this strategy is interesting.

Two of the principal checks on normal cell growth are provided by Rb and p53. Rb, first discovered in retinoblastoma, is involved with cell-cycle regulation. It prevents cells entering S phase until the appropriate growth signal is received. p53, on the other hand, detects damage to DNA and initiates cell suicide by apoptosis. Cells become cancerous, it is thought, if one or both of these sentinel proteins are inactivated. The adenovirus also can cause uncontrolled division, because it is equipped with two genes, *Ela* and *Elb*: *Ela* targets Rb, and *Elb* targets and inactivates p53. It follows that a virus with the *Ela* gene deleted or inactivated would grow only in Rb-deficient cells, and a virus with the *Elb* gene deleted or inactivated would grow only in p53-deficient cells. To date, only the latter idea has been exploited, namely, to produce a virus that replicates only in cells deficient in p53. To the extent that mutant p53 is a hallmark of cancer, this strategy preferentially targets

cancer cells with a cytotoxic virus and spares normal cells. Designing a therapeutic modality that can distinguish between normal and malignant cells has been the Holy Grail of cancer research for decades. Although there is still some debate as to the extent to which these viruses proliferate and kill cells with normal p53, in early clinical trials the systemic inoculation of this vector has produced significant growth suppression of primary head and neck cancer. Again, because only partial responses are evoked, the treatment must be combined with standard radiation or chemotherapy regimes.

### MOLECULAR IMMUNOLOGY (*i.e.*, CANCER VACCINES)

The basis of this approach is to provoke or stimulate a cellular immune response to the invading cancer that is effective against metastatic lesions. Vaccines can be engineered genetically to express cytokines or other molecules known to be important in the generation of immune responses or, alternatively, one of the relatively few known tumor-specific antigens. Vaccination with tumor cells expressing cytokines, such as IL-2, GM-CSF or **IFN-g**, has been shown to induce an **immunologic** response in some animal model systems, resulting in growth arrest (and occasionally cures) of local or metastatic tumors. This approach is not without its problems. First, the molecular requirements for the generation of an immune response that would be capable of causing tumor rejection are not known precisely. Also, in general, antitumor activity is only effective against small tumor burdens. A strategy, still in the development stage but showing some promise, is to combine **molecular immunology with** suicide-gene therapy. The notion here is to use the suicide-gene technique to cause rapid necrosis and to generate large quantities of tumor antigen, in combination with an immune-gene strategy to enhance the immune response to the liberated antigen.

### TUMOR-SUPPRESSOR GENE THERAPY

The closest strategy to real gene therapy is to replace, with a correct copy, a gene, the mutation of which either initiates or substantially alters the malignant phenotype. The goal of such treatment may be to modify cell growth, invasiveness, or metastatic potential as much as to kill the cell.

p53 has received most attention as a target for gene therapy. This is logical, because p53 is the most commonly mutated gene in human cancer and can influence transcription, cell-cycle checkpoints, DNA repair, apoptosis, and angiogenesis.

In several model tumor systems, transduction of cells with wild-type p53 can inhibit growth and angiogenesis or initiate apoptosis. An early phase I clinical trial has been conducted in which lung tumors were injected with a retrovirus carrying p53; this proved to be nontoxic and suppressed tumor growth in six of nine patients.

The factors that greatly limit the field of tumor-suppressor gene therapy are the paucity of known target genes that are causative of the malignant phenotype, or at least are necessary to maintain it, and the fact that more than one genetic change is needed for carcinogenesis.

As with most forms of gene therapy, eradication of treated tumors is a rarity, even in model experimental systems. This is because of the technical difficulty of transducing a sufficiently large proportion of cells within the tumor. The problem is mitigated, but not solved, by an apparent "bystander effect," whereby more cells die than actually are transduced. As might be expected, expression of wild-type p53 in tumor cells can sensitize them to the effects of irradiation, making an additional case for the combined approach to therapy.

### RADIATION-INDUCIBLE GENE LINKED TO A CYTOTOXIC AGENT

The principle here is to combine the physics of radiation-targeting technology

## Radiation Inducible Gene Therapy

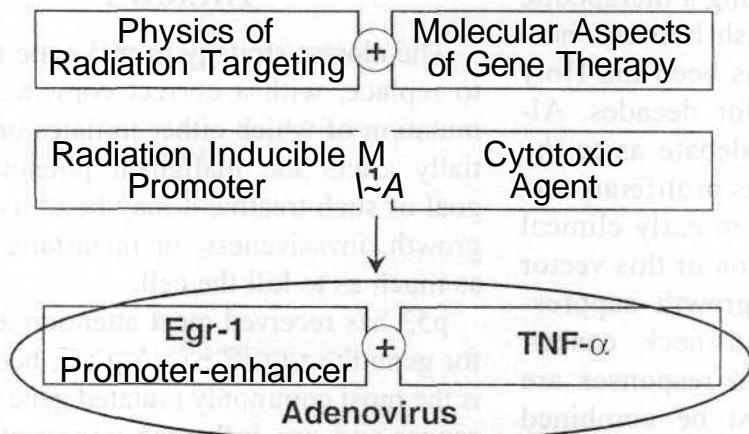


Figure 26.2. Illustrating the general principle of radiation-targeted gene therapy. The idea is to combine the physics of radiation targeting with the molecular aspects of gene therapy. A radiation-inducible promoter, ahead of a cytotoxic agent, and contained in an adenovirus, is injected into the tumor. The promoter is turned on only in the radiation field, thus targeting the therapy.

with the molecular aspects of gene therapy. The general principle is illustrated in Figure 26.2. Specifically, a chimeric gene is created that can be activated by radiation and inserted into the genome of a nonreplicating adenovirus. The chimeric gene involves the human cDNA sequence that encodes tumor necrosis factor (**TNF**)  $\alpha$  and parts of the early growth response gene (*Egr-1*) promoter/enhancer, which is activated by a dose

of radiation of about 0.5 Gy (50 rad). The adenovirus is injected into the tumor, but the TNF $\alpha$  is activated only in the target volume delineated by the radiation field. By this means, total-body toxicity is avoided. Released in the tumor, the TNF $\alpha$  causes vascular destruction as well as apoptosis in tumor cells. The principle is illustrated in Figure 26.3. The key to the success of this strategy is to have radiation-inducible promoters/en-

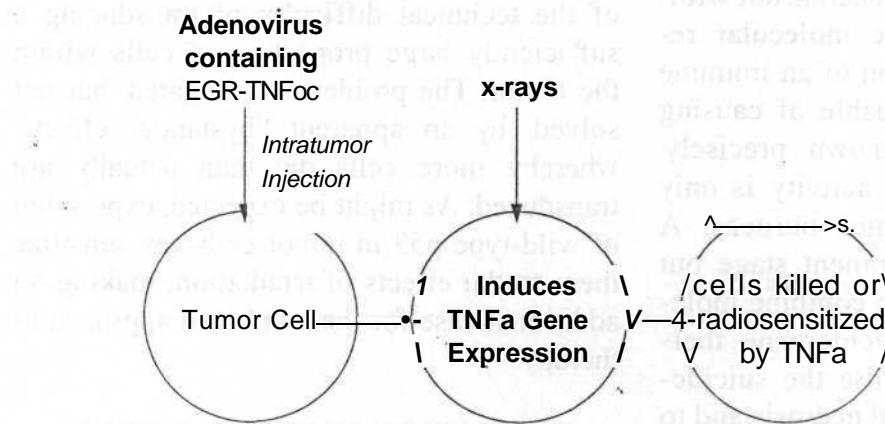


Figure 26.3. The principle of the specific form of radiation-inducible gene therapy, or radiogenetic therapy, developed by Weichselbaum and colleagues. The radiation-inducible gene is the *Egr-1* gene, which if exposed to radiation activates tumor necrosis factor (TNF)  $\alpha$ , which is cytotoxic to tumor cells.

hancers, such as members of the early growth response gene family. These generally respond to a variety of stimuli, including ionizing radiation. The sequences that trigger this radiation response consist of 10 base-pair motifs, known as *CArG elements*, located within the 51 "entrance" regions of the promoter. These elements are activated by reactive oxygen intermediates, the triggering stimuli produced by radiation. *Egr-1* transcripts return to basal levels within 3 hours after irradiation.

Radiogenetic therapy, as it now is called, has shown promise in one model system consisting of human laryngeal tumor cells growing as a xenograft in the hind limbs of immunodeficient nude mice. This single demonstration of the technique may be just a preview of things to come. There is the potential to use a much more radiation-specific promoter than *Egr-1*, with a more effective toxic agent than TNFa. The basic idea is the synthesis of the well-developed physics of radiation-targeting technology with the molecular aspects of gene therapy.

### TARGETING GENE THERAPY

In an attempt to make gene therapy more specific and improve tumor cell killing relative to normal tissue toxicity, a number of possibilities have been explored to target the toxic agent used in gene therapy to particular cells.

For example, many tumors contain regions of hypoxia, and a hypoxia inducible factor has been identified that binds to DNA at specific hypoxia-responsive elements to upregulate transcription of certain genes. Put simply, a hypoxia-sensitive element can be added to the gene-therapy construct so that the prodrug is converted to a cytotoxic agent only in regions of hypoxia, which, it is assumed, occur only in tumors.

Alternatively, tumor-specific promoters can be used. The clearest example is prostate cancer, in which prostate-specific antigen can

be used. There are published reports, too, for specific promoters for hepatoma, breast cancer, and gastric carcinoma. The field is in its infancy, but there is great potential.

### CONCLUSION

The variety of strategies described in this chapter, and lumped together under the heading of gene therapy, all have made significant and impressive progress in the treatment of localized, and even metastatic, cancer in animal models. Some progress has been made in the treatment of cancer in the human, the real problem, but progress is less impressive to date. Significant problems remain to be solved, but the field bears watching.

All strategies discussed share a common problem, namely the difficulty of transducing therapeutic genes into a sufficiently large proportion of tumor cells. For example, a 99% efficiency of infection gives just 2 logs of cell kill; 9 or 10 logs are needed to sterilize the tumor. A mitigating phenomenon is the "bystander effect," whereby more cells are killed than are transduced initially. Bystander effects may act locally via cell-to-cell communication or systemically through an immune-mediated response. In the latter case a local therapy may induce a systemic antitumor response and have an effect on distant metastases.

It would appear logical to combine one or another of the new gene-therapy approaches with standard radiation therapy or chemotherapy. The guiding principle must be to combine modalities that have additive or synergistic effect as far as tumor cell killing is concerned, but for which the limiting normal tissue toxicities are different. The rationale for combining gene therapy with, for example, standard radiotherapy is that a small increment in cell killing from the gene therapy could translate into a large increase in tumor control, because the radiation alone already results in a tumor control on the steep portion of the tumor control-dose relationship.

### SUMMARY OF PERTINENT CONCLUSIONS

- *Gene therapy* is a catch-all term for several very different approaches to cancer therapy.
- All forms of gene therapy rely on vectors to transport a therapeutic agent into cells.
- Viruses are most commonly used as vectors:

*Retroviruses* are convenient, but they infect only dividing cells.

*Adenoviruses* infect both dividing and nondividing cells but invoke an immune response, which can hamper repeat applications.

*Herpesviruses* are bigger, allowing a larger package to be inserted, but are potentially more dangerous.

- Suicide-gene therapy is based on transducing cells with a gene that converts a prodrug into a cytotoxic agent.
- The herpes simplex virus thymidine kinase gene (HSV- $\alpha$ ) plus ganciclovir is an example of suicide-gene therapy.
- The virus containing the gene is injected into the tumor; the ganciclovir is administered systemically.
- The thymidine kinase gene phosphorylates the ganciclovir, converting it into a cytotoxic agent within the tumor.
- There is a substantial *bystander effect*—that is, more cells are killed than transduced initially.
- Suicide-gene therapy has produced growth delay and some cures in animal models.
- More than 30 trials of human tumors have been started.
- A *cytotoxic virus* can be constructed that is engineered to replicate and kill only in cells with mutant p53.
- To the extent that mutant p53 is a hallmark of cancer, this treatment differentiates between normal and cancer cells.
- Growth arrest has been observed in model animal tumors and in early clinical trials.
- Molecular immunology seeks to provoke a cellular immune response against the cancer by injecting a vaccine genetically engineered to express immune stimulatory molecules or tumor-specific antigens.
- Molecular immunology shows some promise in animal models but is generally only effective against small tumor burdens.
- Tumor-suppressor gene therapy is the replacement, with a correct copy, of the mutated gene that initiates or contributes significantly to the malignant phenotype.
- p53 has received the most attention of any gene because it is so commonly mutated in human cancers.
- Phase I clinical trials show some promise in the treatment of lung cancer.
- Tumor-suppressor gene therapy is limited by a lack of information on the target genes that are essential for maintaining the malignant phenotype and the fact that multiple genetic changes are involved.
- Radiation-activated genes combine the physics of radiation-targeting technology with molecular gene therapy.
- The principle of radiation-activated genes is to link a radiation-inducible promoter to a cytotoxic agent; the cytotoxic agent is "turned on" only in the carefully delineated radiation field.
- In the practical case used in a model animal tumor system, the *Egr-1* radiation-inducible promoter is linked to tumor necrosis factor (TNF)  $\alpha$  and packaged in an adenovirus in order to infect the tumor cells.

- There is the potential to use a more radiation-specific promoter gene and a more effective toxic agent.
- There is the possibility of including a promoter that is specific for a particular tumor, for example, prostate or breast.
- Several gene-therapy strategies have made progress in achieving growth reduction in animal model systems and in some human cancers.
- Several forms of gene therapy claim a "bystander" effect; that is, more cells are killed than are transduced initially.
- A problem common to all strategies is to transduce a sufficiently large proportion of tumor cells; growth reduction is common, but cures are rare.
- It is logical to combine gene therapy with radiation therapy or chemotherapy. The hypothesis is that a small increase in cell killing from gene therapy could translate into a large increase in tumor control by conventional therapies.

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## Cytotoxic Anticancer Agents from the Perspective of the Radiation Biologist



radiation can be combined with other modalities such as chemotherapy, immunotherapy, or hyperthermia. For example, radiosensitizers such as carboplatin and cisplatin have been shown to potentiate the cytotoxicity of ionizing radiation. This synergistic effect has led to the development of combined modality therapies for cancer treatment. In addition, the use of radiosensitizers in combination with other treatments such as chemotherapy or immunotherapy can improve patient outcomes. For instance, the use of radiosensitizers in conjunction with chemotherapy has been shown to be effective in the treatment of certain types of cancer, such as head and neck cancer and glioma. These combined approaches have the potential to improve patient survival rates and reduce side effects associated with individual treatments.

The perspective of the radiation biologist is unique in that it focuses on the cellular and molecular mechanisms of radiation-induced damage. This perspective emphasizes the importance of understanding the underlying biological processes that govern the response of cells to radiation. By doing so, researchers can develop more effective and targeted therapies for cancer treatment. For example, the identification of specific genes that are involved in the radiosensitivity of cancer cells can lead to the development of targeted therapies that specifically target these genes. This approach has the potential to improve patient outcomes while minimizing side effects. In addition, the perspective of the radiation biologist can help to identify new targets for cancer treatment, such as specific proteins or pathways that are essential for the survival of cancer cells. By targeting these specific molecules, researchers can develop more effective and targeted therapies for cancer treatment. Overall, the perspective of the radiation biologist is crucial for the development of effective and targeted therapies for cancer treatment.

## Chemotherapeutic Agents from the Perspective of the Radiation Biologist

### BIOLOGIC BASIS OF CHEMOTHERAPY

### CLASSES OF AGENTS AND MODE OF ACTION

### DOSE-RESPONSE RELATIONSHIPS

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### SUMMARY OF PERTINENT CONCLUSIONS

*Alice:* There's no use trying—one can't believe impossible things.

*The Queen:* I dare say you haven't had much practice. Why, sometimes I've believed as many as six impossible things before breakfast.

—*Alice in Wonderland* (Lewis Carroll)

This chapter is included after much thought and some equivocation. It was written in response to numerous requests that chemotherapeutic agents be compared and contrasted with radiation from the perspective of the experimental biologist. Many of the techniques and concepts used in chemotherapy were developed initially by radiation biologists, including quantitative tumor assay systems, the concept of cell cycle, sensitivity changes through the cell cycle, and, particularly, population kinetics. The term **growth fraction**, for example, was coined by a radiation biologist but never assumed the importance in radiotherapy that it has in chemotherapy.

The study of chemotherapeutic agents in the laboratory, as well as in the clinic, is vastly

more complicated than the study of ionizing radiations. For example, dose is more difficult to define or to measure, and time of drug exposure is a critical parameter. Variations in sensitivity through the cell cycle are more dramatic for chemicals than for radiation, assuming essentially an all-or-nothing effect for some agents; there are many more factors involving the milieu that can influence cellular response.

The term **chemotherapy** was coined by Paul Erhlich around the turn of the 20th century to describe the use of chemicals of known composition for the treatment of parasites. Erhlich synthesized an organic arsenic compound that was **effective** against **trypanosome** infections and rabbit syphilis. This was the first manmade chemical effective in the treatment of parasitic disease and rather optimistically named **salvarsan**, which roughly translates to "the savior of mankind." The next milestone was the discovery and clinical use of penicillin in the early years of World War II. Alkylating agents had been developed as a

military weapon by both belligerents in World War I, but it was an explosion in Naples harbor and the exposure of seamen to these agents during World War II that led to the observation that they caused marrow and lymphoid hypoplasia. As a result, they were first tested in humans **with** Hodgkin's disease in 1943 at Yale University.

It has long been known beyond doubt that a single chemotherapeutic drug, used in the appropriate schedule, can cure patients with certain rapidly proliferating cancers. The initial demonstration of this was the use of methotrexate to cure patients with choriocarcinoma and, later, the use of cyclophosphamide for Burkitt's lymphoma.

The next major step forward was the use of combination chemotherapy **in** the treatment of acute lymphocytic leukemia **in** the early 1960s and, subsequently, in the treatment of Hodgkin's disease, diffuse histiocytic lymphoma, and testicular cancer **in** the mid-1970s. These trials verified that multiple non-cross-resistant drugs with different dose-limiting normal-tissue toxicities could be used effectively **in** combination to cure tumors that were not curable with a single agent. The principle of combination therapy then was extended to combined-modality treatment, in which chemotherapy was used in conjunction with surgery or radiotherapy, or both, to cure tumors such as pediatric sarcomas.

Today a wide variety of antineoplastic agents are used routinely **in** clinical oncology. Drug-induced cures are claimed for choriocarcinoma, acute lymphocytic leukemia of childhood, other childhood tumors, Hodgkin's disease, certain non-Hodgkin's lymphomas, and some **germ** cell tumors of the testes. Other evidence suggests that chemotherapeutic agents given in an "adjuvant" setting for clinically inapparent micrometastatic disease may prolong disease-free survival and possibly effect cure of breast cancer and osteogenic sarcoma.

The chemotherapy of cancer is the treatment of metastatic disease. With the exception of some leukemias and lymphomas, its function is one of retrieval—to treat and possibly to control a cancer that has become sys-

temic and out of control. There are 13 types of cancer for which cures are claimed by chemotherapy; this accounts for about 10% of all cancers. For comparison, the proportion of cancer patients cured by radiation therapy often is claimed to be about 12.5%. This comes from the so-called  $1/2 \times 1/2 \times 1/2$  rule; that is, one half of all cancer patients receive radiation therapy, one half of those treated are treated with intent to cure, and one half of those treated definitively are cured.

The bad public image of chemotherapy results in large part to the toxicity to normal tissue resulting from multidrug protocols used to induce remissions and achieve tumor cure. The lack of tumor-specific agents carries the burden of damage to self-renewing normal tissues, such as the gut, bone marrow, and scalp. Until drugs can be developed that discriminate better between normal and neoplastic tissue, chemotherapy will continue to be a primitive form of cancer therapy—using the proverbial sledgehammer to crack a nut.

## BIOLOGIC BASIS OF CHEMOTHERAPY

Almost all anticancer drugs work by affecting DNA synthesis or function, and they usually do not kill resting cells unless such cells divide soon after exposure to the drug. Consequently, the effectiveness of anticancer drugs is limited by the growth fraction of the tumor—by the fraction of cells **in** active cycle. Rapidly growing neoplasia with a short cell cycle, a large proportion of cells in S phase, and therefore a large growth fraction are more responsive to chemotherapy than large tumor masses, in which the growth fraction is small. There is a strong tendency for growth fraction to decrease as tumor size increases, at least in experimental animal tumors.

Agents that are mainly effective during a particular phase of the cell **cycle**, such as S phase or M phase, are said to be **cell-cycle specific**, or **phase specific**. Those whose action is independent of the position of the cell in the cycle are said to be **cell-cycle nonspecific** or **phase nonspecific**. Figure 27.1 illustrates two

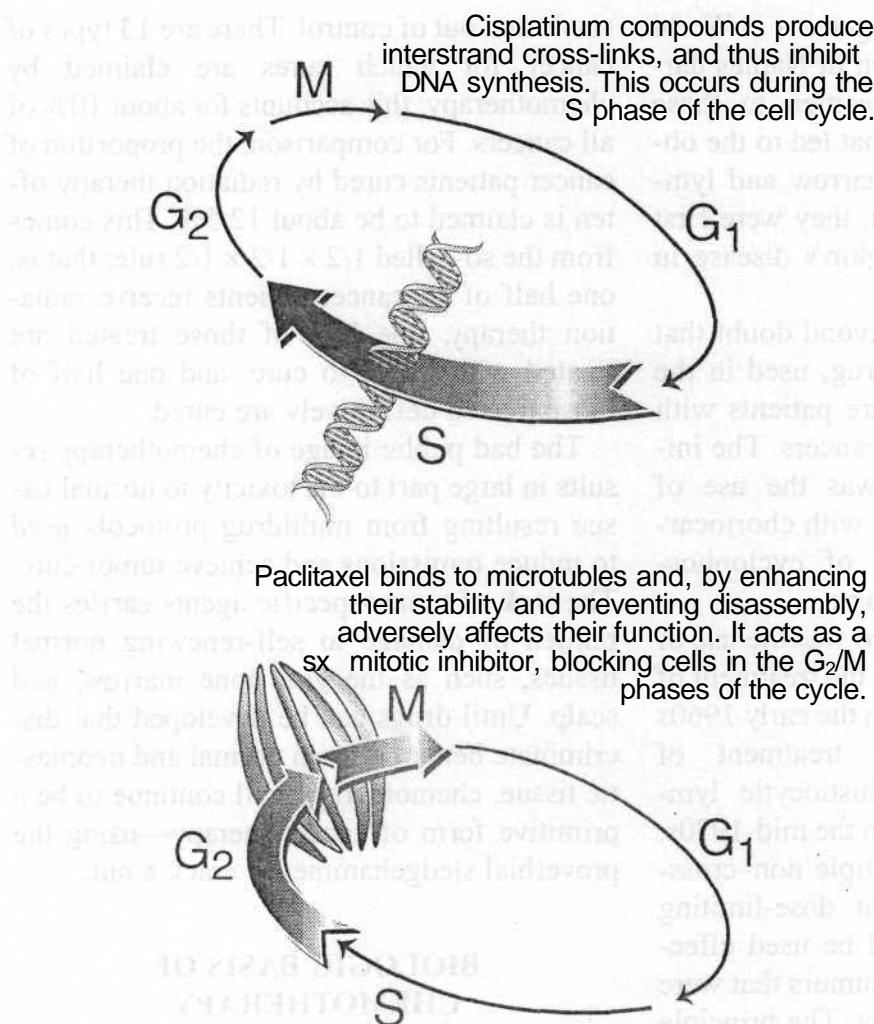


Figure 27.1. Two contrasting chemotherapy agents that are both cell-cycle phase specific but produce their effects at quite different phases. C/s-platinum compounds produce DNA interstrand cross-links that inhibit DNA synthesis; this occurs in the S phase. Taxanes bind to microtubules and, by enhancing their stability and preventing disassembly, adversely affect their function. Taxanes act as mitotic inhibitors, blocking cells in the G<sub>2</sub>/M phase of the cycle, and, if the concentration is sufficient, killing them in this phase.

contrasting cell-cycle specific drugs. **Cis-platin** compounds produce interstrand cross-links and thus inhibit DNA synthesis; this occurs in S phase. Taxanes bind to microtubules and, by enhancing their stability and preventing disassembly, adversely affect their function. They act as mitotic **inhibitors**, blocking cells in the **G<sub>2</sub>/M** phase of the cell cycle.

Agents that are most effective against cells **in** S phase are relatively ineffective against cell populations that turn over slowly and have large proportions of **dormant** cells. On the other hand, the action of alkylating agents and other **drugs interacting primarily with** macromolecular DNA is largely independent of the phase of

the cell cycle, and they may be effective against tumors **with** relatively low proliferative activity.

The other side of the coin is that the selective normal tissue toxicity of anticancer drugs is reflected in cytotoxic effects on stem cells of the intestinal epithelium or hematopoietic stem cells, which have high growth fractions.

Although many clinical oncologists claim that their thinking has been influenced by research on tumor-growth kinetics, it is hard to point to clear advances in treatment that may be attributed to anything more than inspired clinical experimentation. This may be because the study of growth kinetics in human tumors is still in its infancy.

The effectiveness of at least some chemotherapeutic agents is dependent on the presence or absence of molecular oxygen, in much the same way as x-rays. This is not surprising, at least for drugs whose action is mediated by free radicals.

### CLASSES OF AGENTS AND MODE OF ACTION

Most commonly used chemotherapeutic agents fall into one of three classes: alkylating agents, antibiotics, or antimetabolites. Some of the most important and most widely used agents, however, make up a fourth mixed class that can only be labeled "miscellaneous." This includes the platinum complexes, procarbazine, and the vinca alkaloids.

An attempt to summarize the classification of drugs is presented in Table 27.1. A few of the most commonly used agents are described briefly, with emphasis on their characteristics

and mechanism of action. A thorough discussion of their clinical usefulness is outside the scope of this book.

### Alkylating Agents

The alkylating agents are highly reactive compounds with the ability to substitute alkyl groups for hydrogen atoms of certain organic compounds, including DNA. There are five classes of alkylating agents:

1. Nitrogen mustard derivatives, such as cyclophosphamide, chlorambucil, and melphalan
2. Ethylenimine derivatives, such as thiotepa
3. Alkyl sulfonates, such as busulfan
4. Triazine derivatives, such as dacarbazine
5. Nitrosoureas, including BCNU (carmustine), CCNU (lomustine), and methyl CCNU

Most of these drugs contain more than one alkylating group and therefore are considered

TABLE 27.1. *Classes of Chemotherapeutic Agents*

Class of Compound	Examples	Examples of Diseases in which Drugs are Useful
Alkylating agents	Nitrogen mustard, chlorambucil, cyclophosphamide, busulfan	Lymphomas, many solid tumors, chronic leukemia, multiple myeloma
Antimetabolites	Methotrexate 6-Mercaptopurine Cytarabine 5-Fluorouracil	Acute leukemia Choriocarcinoma, head and neck cancer
Antibiotics	Dactinomycin Doxorubicin Bleomycin	Carcinoma of breast, carcinoma of gastrointestinal tract Wilms' tumor, choriocarcinoma
Plant alkaloids	Vincristine Vinblastine	A wide spectrum of tumors Lymphomas, testicular carcinoma Acute leukemia, lymphomas Reticuloendothelial malignancy lymphomas, testicular carcinoma
Adrenocorticosteroids	Prednisone	Lymphocytic leukemias, lymphomas, carcinoma of breast
Other steroid hormones	Estrogens Androgens	Carcinoma of prostate Carcinoma of breast
Antiestrogens	Progesterins Tamoxifen	Carcinoma of endometrium Carcinoma of breast
Enzymes	L-Asparaginase	Acute lymphatic leukemias, lymphomas
Miscellaneous agents		
Methylhydrazine	Procarbazine	Lymphomas
Nitrosoureas	BCNU CCNU	Lymphomas Brain tumors, many solid tumors
Hydroxyurea	Hydroxyurea	Chronic and acute leukemias
C/s-platinum	C/s-platinum	Testicular cancer
Taxanes	Paclitaxel Docetaxel	Breast cancer

to be **polyfunctional alkylating agents**. The nitrosoureas and dacarbazine have mechanisms and cytotoxicity over and above their ability to alkylate nucleic acids. As a class, alkylating agents are considered to be cell-cycle nonspecific.

Nitrogen mustard is the prototype for three other useful alkylating agents: cyclophosphamide, chlorambucil, and melphalan. These drugs are given intravenously and interact rapidly with cells *in vivo*, producing their primary effect in seconds or minutes. By contrast, cyclophosphamide (Cytoxan) is inert until it undergoes biotransformation in the liver. Disappearance of injected cyclophosphamide from the plasma is biexponential, with an average half-life of 4 to 6.5 hours. Like all useful alkylating agents, cyclophosphamide produces toxicity **in** rapidly proliferating **normal** tissues. Chlorambucil (Leukeran) is an aromatic derivative of nitrogen mustard and is the slowest acting alkylating agent in general use. Melphalan (Alkeran, L-PAM) is a phenylalanine derivative of nitrogen mustard.

The nitrosoureas are a group of lipophilic alkylating agents that undergo extensive biotransformation *in vivo*, leading to a variety of biologic effects, including alkylation, carbamylation, and inhibition of DNA repair. The multiple mechanisms of action may explain why the nitrosoureas generally lack cross-resistance **with** other alkylating agents. These compounds are very lipid-soluble and readily cross the blood-brain barrier. They disappear from plasma rapidly, but their metabolites may persist for days.

#### Antibiotics

The clinically useful antibiotics are natural products **of** various strains of the soil fungus *Streptomyces*. They produce their tumoricidal effects by directly binding to DNA, and so their major inhibiting effects are on DNA and RNA synthesis. As a class, these drugs behave as cell-cycle nonspecific agents. Doxorubicin (Adriamycin) and daunomycin are closely related anthracycline antibiotics. After intravenous injection, both drugs undergo extensive

bioreduction in the liver to active and inactive metabolites, are bound extensively in tissues, and persist in plasma for prolonged periods. Neither drug crosses the blood-brain barrier to any appreciable extent. Both doxorubicin and daunorubicin are highly toxic drugs, producing a variety of severe reactions; the major limiting toxicity, however, is cardiac damage.

Dactinomycin (Actinomycin D) inhibits DNA-primed RNA synthesis by intercalating with the guanine residues of DNA; at higher concentrations it also inhibits DNA synthesis. The net effect is cell-cycle nonspecific cytotoxicity. Dactinomycin must be administered intravenously. Its important longer plasma half-life is about 36 hours, and the drug is extensively bound to tissues.

Bleomycin sulfate (Blenoxane) affects cells by directly binding to DNA, resulting **in** reduced synthesis of DNA, RNA, and proteins. It also can lead to single-strand DNA breaks. Drugs acting by intercalation appear to augment the cytotoxic effects of bleomycin, as do x-rays and chemicals that generate superoxide radicals. Bleomycin is considered cell-cycle nonspecific. It is more damaging to nonproliferating than to most proliferating cells.

Mitomycin C (Mutamycin) is an extremely toxic antitumor antibiotic. Unlike most other antibiotics, it is activated *in vivo* to a bifunctional or trifunctional alkylating agent. It is cell-cycle nonspecific and is considerably more toxic to hypoxic than to aerated cells. Mitomycin C almost always is administered intravenously; it is cleared rapidly from the plasma, with a half-life of 10 to 15 minutes, primarily by metabolism in the liver. It does not appear to cross the blood-brain barrier. The major toxicity of mitomycin C is myelosuppression.

#### Vinca Alkaloids

Some of the most useful antineoplastic agents are produced from plants. Vincristine sulfate (Oncovin) and vinblastine sulfate (Velban) are alkaloids produced from the common periwinkle plant. The clinically useful alkaloids are large complex molecules that exert their major antitumor effect by binding to cellular

microtubular proteins and inhibiting microtubule polymerization. Because these are essential compounds of the mitotic spindle of dividing cells, this binding leads to mitotic arrest.

### Taxanes

The toxicity of products of the yew tree has been known for thousands of years. For example, essentially every rural village in England has a huge spreading yew tree, dating from medieval times when the archer's weapon, the long bow, was fabricated from the wood of the yew—but the tree always is found in the walled-in church cemetery, because the leaves or bark are toxic to browsing livestock. This toxicity is caused by a class of compounds known as taxanes. For those who see chemotherapy for cancer as simply the latest round in the never-ending battle between plants and animals, taxanes do indeed provide the perfect example.

Paclitaxel is the prototype of a new class of antineoplastic agents, the taxanes, that targets the microtubules. It is a natural product, isolated from the bark of the western yew, *Taxus brevifolia*. Docetaxel is a largely synthetic derivative.

Taxanes are potent microtubule-stabilizing agents and promoters of microtubule assembly. This is in contrast to agents such as the vinca alkaloids and colchicine that bind to tubulin, the subunit of microtubules, and inhibit microtubule formation. The taxanes block or prolong the transit time of cells in the G<sub>2</sub>/M phase of the cell cycle. The inability of these cells to pass through the G<sub>1</sub> and M phases of the cycle results from the inability of these cells to form a competent mitotic spindle or to disassociate a drug-treated spindle.

In addition to multiple *in vitro* studies from the early 1970s on, human studies have demonstrated the ability of taxanes to increase the mitotic index in a variety of normal tissues *in vivo* while the two taxanes *in* clinical use, Taxol (paclitaxel) and Taxotere (docetaxel), have demonstrated significant levels of activity in a broad range of human tumors. The taxanes are of particular interest to radiobiologists because

of the way in which they interact **with** radiation, as is described subsequently.

### Antimetabolites

The antimetabolites are analogues of normal metabolites required for cell function and replication. They may interact with enzymes and damage cells by

1. *Substituting* for a metabolite normally incorporated into a key molecule
2. *Competing* successfully with a normal metabolite for occupation of the catalytic site of a key enzyme
3. *Competing* with a normal metabolite that acts at an enzyme regulatory site to alter the catalytic rate of the enzyme

Methotrexate is a folic-acid antagonist. It works by competing for the folate-binding site of the enzyme dihydrofolate reductase. This results in decreased synthesis of thymidine and purine nucleotides. The cytotoxicity of methotrexate can be reversed by leucovorin, which is converted readily to other **forms** of reduced folate within the cell and which then can act as methyl donors for a variety of biochemical reactions. The use of high-dose methotrexate with leucovorin rescue is based on the pharmacology of the two drugs, with the possibility of a differential effect between tumors and normal tissues in their ability to transport the two drugs across cell membranes. How true this differential effect turns out to be is another matter.

### 5-Fluorouracil

5-Fluorouracil is a structural analogue of the DNA precursor thymine. It works primarily as an irreversible inhibitor of the enzyme thymidylate synthetase, but only after intracellular conversion to the active metabolite. It also is degraded by the liver and some other tissues. As a single agent, 5-fluorouracil is most useful in the treatment of carcinoma of the breast and gastrointestinal tract. The degradative enzymes are found in high concentrations in the gut but not *in* colonic carcinomas, and it has been suggested that this may explain in part the susceptibility of this tumor to 5-fluorouracil.

### Nucleoside Analogues

A variety of nucleoside analogues have been synthesized and tested for antineoplastic properties. They are transported readily into rapidly dividing cells and activated by the single metabolic step of phosphorylation. Two analogues of cytosine are useful in cancer chemotherapy.

Cytarabine (cytosine arabinoside) is an analogue of deoxycytidine in which the sugar moiety is altered. The active form of cytarabine is the triphosphate that functions as a competitive inhibitor of DNA polymerase. Cytarabine is cell-cycle specific and in clinical practice almost always is used in combination with other drugs in the treatment of acute myeloid leukemia.

5-Azacytidine contains a single nitrogen substitution in the pyrimidine ring of cytidine. It undergoes a sequence of biotransformations similar to cytarabine, with the ultimate formation of an active triphosphate. The major biochemical effect of 5-azacytidine is believed to be the inhibition of the processing of large molecular weight species of RNA, with less important effects on DNA and protein synthesis. Like cytarabine, it is cell-cycle specific.

### Miscellaneous Agents

#### Procarbazine

Procarbazine is a hydrazine derivative that must undergo biotransformation before it can exert its cytotoxic effects. The precise mechanism of action is not clear, because it interferes with a wide variety of biochemical processes. Procarbazine is well absorbed from the gastrointestinal tract and is cleared from the plasma with a half-life of about 10 minutes. The drug freely crosses the blood-brain barrier. It is used primarily in the treatment of advanced Hodgkin's disease.

#### Hydroxyurea

Hydroxyurea first was synthesized as long ago as 1869 and was found to be bone-marrow suppressive in 1928. It was not used in the treatment of cancer until the 1960s. It acts as an in-

hibitor of ribonucleotide reductase, an enzyme essential to DNA synthesis, and is consequently specifically cytotoxic to cells in the S phase of the cell cycle. In experimental biology hydroxyurea is used to synchronize cells, because in addition to killing S phase cells, it causes survivors to pile up at a block at the G<sub>1</sub>-S interface. Clinically, hydroxyurea is used primarily in the treatment of chronic myeloid leukemia.

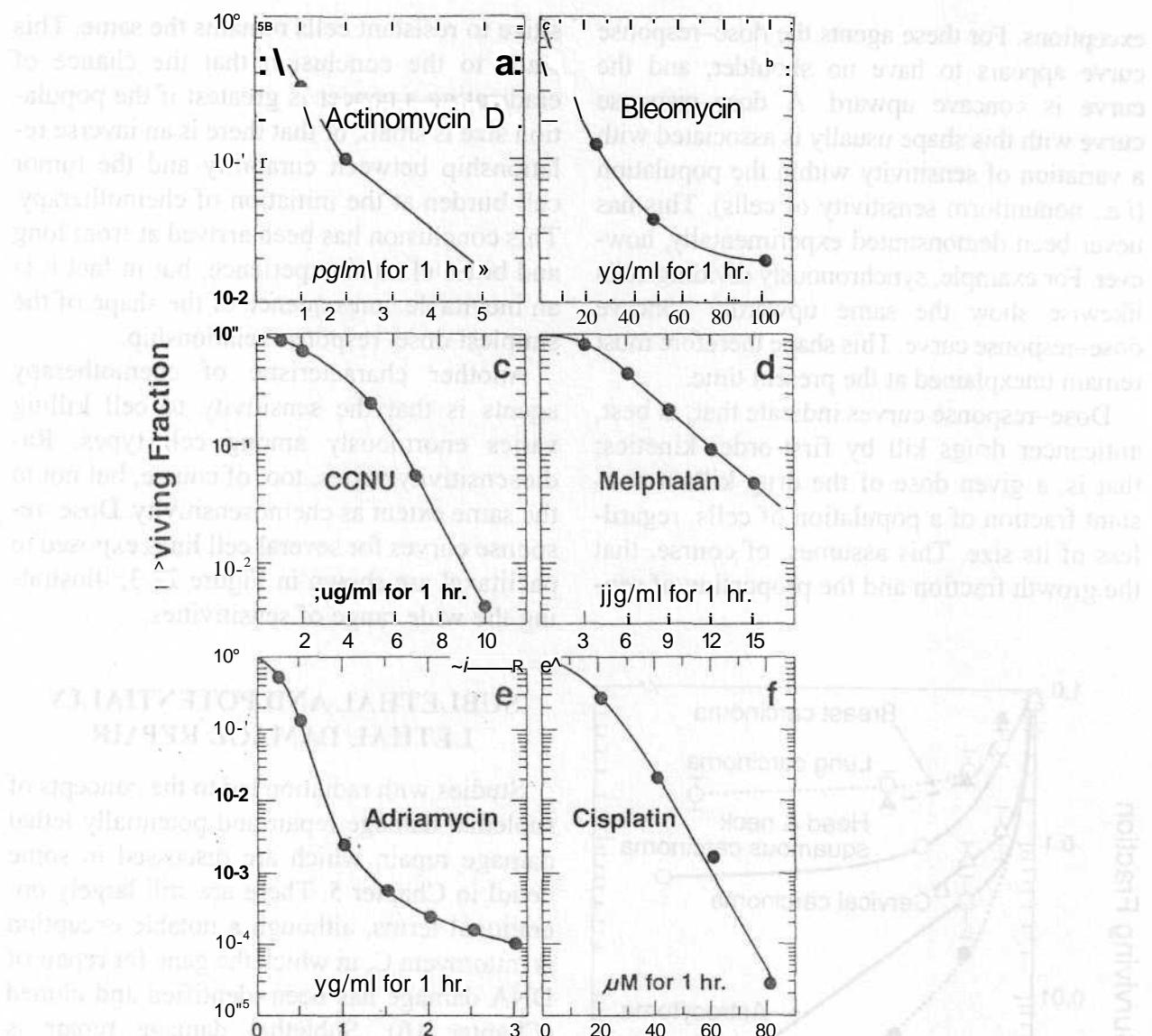
#### Cis-platinum

Structurally, cis-platinum (ds-dichlorodiammineplatinum) is an inorganic complex formed by an atom of platinum surrounded by chlorine and ammonium ions in the *cis* position of the horizontal plane. Cis-platinum bears a resemblance to the bifunctional alkylating agents based on nitrogen mustard. It inhibits DNA synthesis to a greater extent than the synthesis of RNA or protein. It binds to DNA, causing both interstrand and intrastrand cross-linking.

Qs-platinum is cell-cycle nonspecific. Its isomer, fra/w-platinum, is much less cytotoxic, presumably because of the different way that it cross-links to DNA. There is some evidence that c/s-platinum is more toxic to hypoxic than to aerated cells; that is, that it is a hypoxic cell radiosensitizer, though not as powerful in this regard as the nitroimidazoles.

### DOSE-RESPONSE RELATIONSHIPS

Dose-response relationships have been produced for a wide range of chemotherapeutic agents using techniques developed initially for radiation, although much less effort has been expended on fitting data to models than has been the case for ionizing radiations. From even a cursory examination of the data, however, it is evident that—with some clear exceptions—the shape of the survival curve is unremarkable and reminiscent of that of survival curves for ionizing radiations. If surviving fraction is plotted on a log scale against drug dose on a linear scale, the dose-response curve has an initial shoulder followed by a region that becomes steeper and straighter (Fig. 27.2). The antibiotics doxorubicin, bleomycin, and dactinomycin are clear



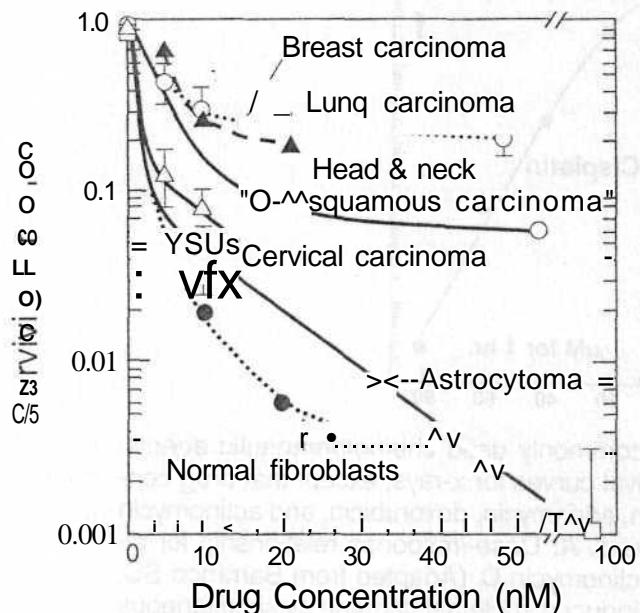
**Figure 27.2.** Dose-response relationships *in vitro* for six commonly used chemotherapeutic agents. Note the diverse shapes. Many have shapes similar to survival curves for x-rays, except that drug concentration replaces absorbed dose. The antibiotics bleomycin, adriamycin, doxorubicin, and actinomycin D have dose-response relationships that are concave upward. A: Dose-response relationship for dividing CHO cells treated for 1 hour with graded doses of actinomycin D. (Adapted from Barranco SC, Fluorney DR: Modification of the response to actinomycin-D-induced sublethal damage by simultaneous recovery from potentially lethal damage in mammalian cells. Cancer Res 36:1634-1640, 1976, with permission.) B: Dose-response relationship for plateau-phase CHO cells treated for 1 hour with graded doses of bleomycin. (Adapted from Barranco SC, Novak JKJ, Humphrey RM: Response of mammalian cells following treatment with bleomycin and 1,3-BTS(2 chloroethyl)-1-nitrosourea during plateau phase. Cancer Res 33:691-694, 1973, with permission.) C: Dose-response relationship for CHO cells treated for 1 hour with graded doses of CCNU. (Adapted from Barranco SC: In vitro responses of mammalian cells to drug-induced potentially lethal and sublethal damage. Cancer Treat Rep 60:1799-1810, 1976, with permission.) D: Dose-response relationship for human lung cancer cells exposed for 1 hour to graded doses of melphalan. (Unpublished data, courtesy of Dr. Laurie Roizin-Towle.) E: Dose-response relationship for V79 Chinese hamster cells exposed for 1 hour to graded doses of doxorubicin. (Adapted from Belli JA, Piro AJ: The interaction between radiation and Adriamycin damage in mammalian cells. Cancer Res 37:1624-1630, 1975, with permission.) F: Dose-response relationship for V79 Chinese hamster cells exposed for 1 hour to graded doses of c/s-platinum. (Unpublished data, courtesy of Dr. Laurie Roizin-Towle.)

exceptions. For these agents the dose-response curve appears to have no shoulder, and the curve is concave upward. A dose-response curve with this shape usually is associated with a variation of sensitivity within the population (*i.e.*, nonuniform sensitivity of cells). This has never been demonstrated experimentally, however. For example, synchronously dividing cells likewise show the same upwardly concave dose-response curve. This shape therefore must remain unexplained at the present time.

Dose-response curves indicate that, at best, anticancer drugs kill by first-order kinetics; that is, a given dose of the drug kills a constant fraction of a population of cells, regardless of its size. This assumes, of course, that the growth fraction and the proportion of sen-

sitive to resistant cells remains the same. This leads to the conclusion that the chance of eradicating a cancer is greatest if the population size is small, or that there is an inverse relationship between curability and the tumor cell burden at the initiation of chemotherapy. This conclusion has been arrived at from long and bitter clinical experience, but in fact it is an inevitable consequence of the shape of the simplest dose-response relationship.

Another characteristic of chemotherapy agents is that the sensitivity to cell killing varies enormously among cell types. Radiosensitivity varies, too, of course, but not to the same extent as chemosensitivity. Dose-response curves for several cell lines exposed to paclitaxel are shown in Figure 27.3, illustrating the wide range of sensitivities.



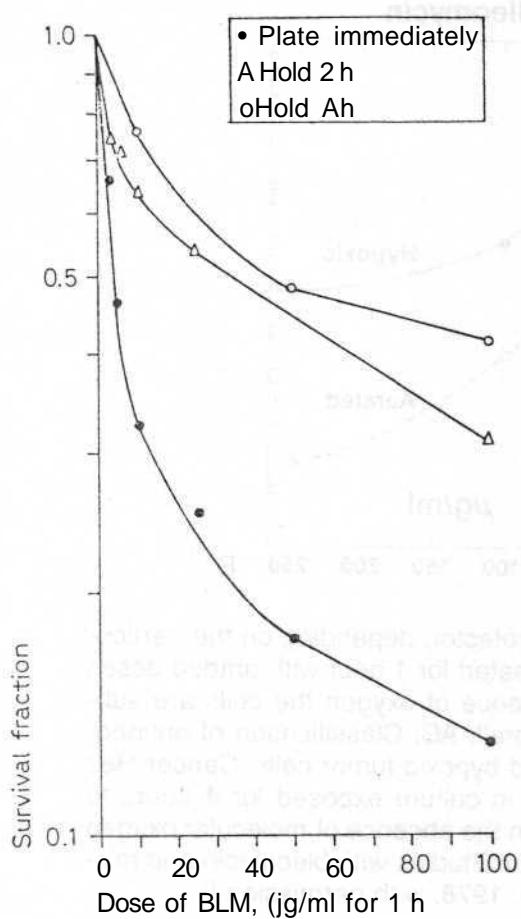
**Figure 27.3.** Illustrating the wide variability in sensitivity to taxanes. All of the data refer to cells of human origin exposed for 24 hours. (Data for two cervical carcinoma lines and an astrocytoma line from Geard CR, Jones JM, Schiff PB: Taxol and radiation. J Natl Cancer Inst Monogr 15: 89-94, 1993; data for a head-and-neck squamous carcinoma line from Leonard CE, Chan DC, Chou TC, Kumar R, Bunn PA: Paclitaxel enhances in vitro radiosensitivity of squamous carcinoma cell lines of the head and neck. Cancer Res 56:5198-5204, 1996; data for a lung carcinoma line [A545] from Lieberman J: Cancer Res 53:2066, 1993; data for a breast carcinoma line [MCF-7] from Lieberman J: Br J Cancer 68:1104, 1993.)

#### SUBLETHAL AND POTENTIALLY LETHAL DAMAGE REPAIR

Studies with radiation led to the concepts of sublethal damage repair and potentially lethal damage repair, which are discussed in some detail in Chapter 5. These are still largely operational terms, although a notable exception is mitomycin C, in which the gene for repair of DNA damage has been identified and cloned (Chapter 16). Sublethal damage repair is demonstrated by an increase in survival if a dose of radiation (or other cytotoxic agent) is divided into two or more fractions separated in time. There is a tendency for the extent of sublethal damage repair to correlate with the shoulder of the acute dose-response curve, but this is not necessarily always true. Repair of potentially lethal damage is manifest as an increase in survival if cells are held in a nonproliferative state for some time after treatment.

Similar studies have been performed with a variety of chemotherapeutic agents. The results are not as clearcut as for radiation, and there is much greater variability between different cell lines.

Potentially lethal damage repair is a significant factor in the antibiotics bleomycin and doxorubicin. Data for bleomycin are shown in Figure 27.4. Potentially lethal damage repair



**Figure 27.4.** Potentially lethal damage repair (PLDR) in cultured Chinese hamster cells treated with bleomycin. An increase in survival is observed, interpreted as PLDR, if cells are held in depleted medium for 2 to 4 hours after the drug treatment. (From Barranco SC, Humphrey RM: Response of mammalian cells to bleomycin-induced potentially lethal and sublethal damage. Prog Biochem Pharmacol 11:78-92, 1976, with permission.)

also is seen after treatment with dactinomycin. Sublethal damage repair is essentially absent with all of these drugs.

No potentially lethal or sublethal damage repair is seen with nitrosourea, even though the dose-response curves for single doses have substantial shoulders. The breakdown products of the nitrosoureas are known to inhibit DNA repair, and this may be a contributing factor.

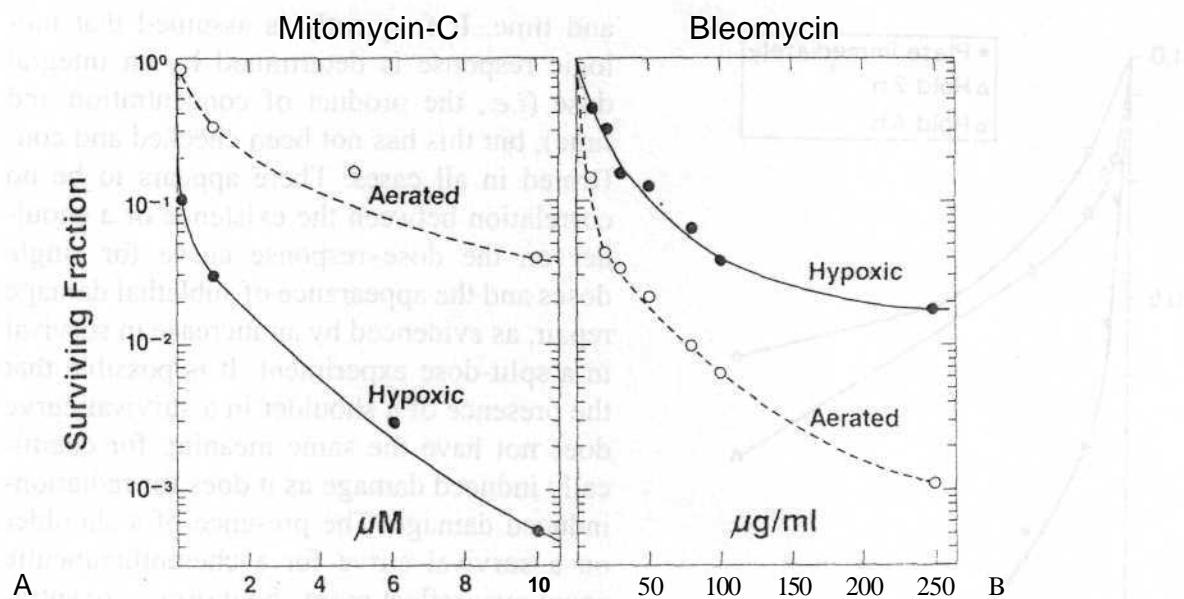
Studies of repair of sublethal damage with drugs are complicated, because if a split-dose study is performed, decisions must be made about the equivalence of drug concentration

and time. It frequently is assumed that biologic response is determined by an integral dose (*i.e.*, the product of concentration and time), but this has not been checked and confirmed **in** all cases. There appears to be no correlation between the existence of a shoulder on the dose-response curve for single doses and the appearance of sublethal damage repair, as evidenced by an increase in survival **in** a split-dose experiment. It is possible that the presence of a shoulder **in** a survival curve does not have the same meaning for chemically induced damage as it does for radiation-induced damage. The presence of a shoulder on a survival curve for a chemotherapeutic agent may reflect more about drug concentrations and the time required for entry of the drug into the cells and interaction with a target molecule than it does about the accumulation and repair of sublethal damage.

### THE OXYGEN EFFECT AND CHEMOTHERAPEUTIC AGENTS

The importance of the oxygen effect for cell killing by radiation is discussed in Chapter 6. It has been known for half a century that the presence or absence of molecular oxygen has a dramatic influence on the proportion of cells surviving a given dose of x-rays. Only in more recent years has the influence of oxygen on the cytotoxicity resulting from chemotherapeutic agents been studied. It is certainly more complicated than for ionizing radiations.

Some agents, such as bleomycin, are more toxic to oxygenated cells than to chronically hypoxic cells. Dose-response curves for cells exposed to graded concentrations of bleomycin **in** the presence or absence of oxygen are shown in Figure 27.5. At high concentrations of the drug there is an extra log of cell killing if oxygen is present, compared with hypoxic conditions. Other examples of agents that are more toxic to aerated than to hypoxic cells are procarbazine, dactinomycin, and vinristine. It should come as no surprise to those used to x-rays that oxygen is a factor in the response of cells to any chemotheapeutic agent



**Figure 27.5.** Molecular oxygen can be either a sensitizer or a protector, depending on the particular chemotherapeutic agent. A: Survival curves for EMT6 cells treated for 1 hour with graded doses of mitomycin C under aerated or hypoxic conditions. In the absence of oxygen the cells are substantially more sensitive. (Data from Teicher BA, Lazo JS, Sartorelli AC: Classification of antineoplastic agents by their selective toxicities toward oxygenated and hypoxic tumor cells. *Cancer Res* 41:73-81, 1981.) B: Survival curves for Chinese hamster cells in culture exposed for 4 hours to graded doses of bleomycin under aerated or hypoxic conditions. In the absence of molecular oxygen the cells are more resistant. (Adapted from Roizin-Towle L, Hall EJ: Studies with bleomycin and misonidazole on aerated and hypoxic cells. *Br J Cancer* 37:254-260, 1978, with permission.)

in which the mechanism of cell killing is mediated by free radicals.

By contrast, agents such as mitomycin C are substantially more toxic to hypoxic than to aerated cells (Fig. 27.5), because the drug undergoes bioreduction in the absence of oxygen. The same is true, of course, of tirapazamine, which is discussed in Chapter 25.

A third group of drugs, including 5-fluorouracil, methotrexate, cis-platinum, and the nitrosoureas, appear to be equally cytotoxic to aerated or hypoxic cells. This oversimplified classification only holds true if the level or duration of the hypoxia is not sufficient to disturb the movement of cells through the cell cycle. Table 27.2 is a summary of the classifi-

**TABLE 27.2. Classification of Antineoplastic Agents Based on Cellular Oxygenation**

Preferential Toxicity to Aerobic Cells	Preferential Toxicity to Hypoxic Cells	Minimal or No Selectivity Based on Cellular Oxygenation
Bleomycin	Mitomycin-C	5-Fluorouracil <sup>a</sup>
Procarbazine	Doxorubicin	Methotrexate <sup>a</sup>
Streptonigrin	Misonidazole, metronidazole	Cisplatin
	Etanidazole	
	Tirapazamine	
	RB6145	
Dactinomycin	5-thio-D-glucose, 2-deoxy-D-glucose	BCNU, CCNU

<sup>a</sup>These conclusions are based on experiments in which hypoxic cells were still capable of DNA synthesis and cellular replication. These agents have cytotoxic effects primarily on cells in the S phase of the cell cycle. Thus, in hypoxic cells that are blocked in their progression through the cell cycle or cycling slowly, agents such as these that act on the S phase of the cell cycle would be expected to be relatively noncytotoxic.

Based on Teicher BA, Lazo JS, Sartorelli AC: Classification of antineoplastic agents by their selective toxicities toward oxygenated and hypoxic tumor cells. *Cancer Res* 41:73-81, 1981, with permission.

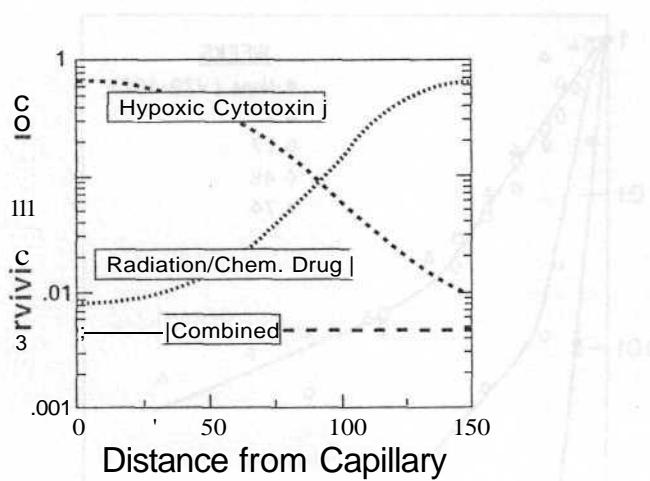
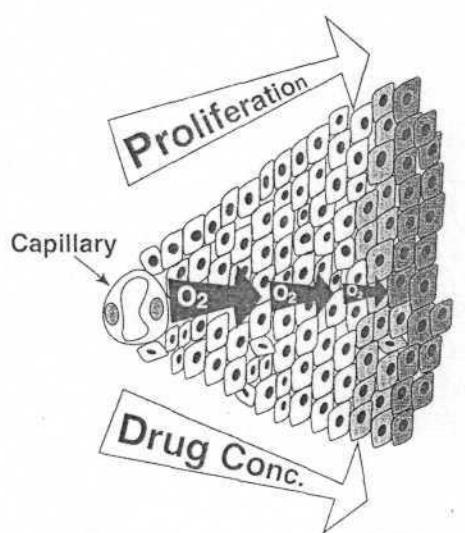
cation of antineoplastic agents based on the effect of the presence or absence of molecular oxygen.

### RESISTANCE TO CHEMOTHERAPY AND HYPOXIC CYTOTOXINS

Tumor cells protect themselves from changes in the microenvironment resulting from a decrease in both nutrients and oxygen by reducing macromolecular synthesis and inducing genes that promote angiogenesis and tissue remodeling. This is discussed in some detail in the chapter on the oxygen effect (Chapter 6). The ability of malignant cells to survive fluctuations in oxygen tension is important in the context of cancer therapy, because it is well documented that tumors that are hypoxic are aggressive and respond poorly to all forms of treatment.

The shortage of oxygen in hypoxic cells renders them refractory to killing by ionizing radiations that require oxygen to "fix" the damage produced in DNA by the hydroxyl radicals; it is equally true that cells may be refractory to killing by "radiation-mimetic drugs" that operate *via* a free radical mechanism. Cessation of cell division and loss of apoptotic potential (cell suicide), however, in hypoxic cells are likely to be more important reasons why hypoxic cells are resistant to chemotherapy. The most important cells that are hypoxic are located remote from capillaries. This is illustrated in Figure 27-6.

The most effective way to partly circumvent the problem introduced by hypoxia in reducing the efficacy of chemotherapy is to introduce a bioreductive drug or hypoxic cytotoxin that is most effective at reduced oxygen concentrations. This is described in



**Figure 27-6.** Illustrating why the microenvironment in a tumor may lead to resistance to chemotherapy agents, and how the addition of a hypoxic cytotoxin may remedy the situation. **Left:** Oxygen diffuses from a capillary, but the concentration falls with distance because of respiration in the mass of tumor cells. This leads to a region of hypoxia and a region of anoxia. Chemotherapy agents must also diffuse through a tumor from capillaries. The effect of the agent decreases with distance from the capillary because the drug concentration falls because of metabolism, proliferation decreases with distance from a capillary because of lack of oxygen, and cells that are not dividing are resistant to many chemotherapy agents. **Right:** The fraction of cells surviving a treatment with radiation or most chemotherapy agents rises with distance from a capillary because both are less effective in hypoxic nondividing cells. This can be balanced by the addition of a hypoxic cytotoxin, such as tirapazamine, which only becomes effective in regions of reduced oxygen concentration. Consequently the fraction of cells surviving after a hypoxic cytotoxin decreases with distance from a capillary; that is, more cells are killed. (Adapted from Brown JM, Siim BG: Semin Radiat Oncol 6:22-36, 1996, with permission.)

more detail in the chapter on radiosensitizers and bioreductive drugs (Chapter 25). Mitomycin C and its derivatives have been used for this purpose for many years. A more recently developed drug is tirapazamine, which already has been used successfully in combination with m-platinum in the therapy of advanced lung cancer.

### DRUG RESISTANCE

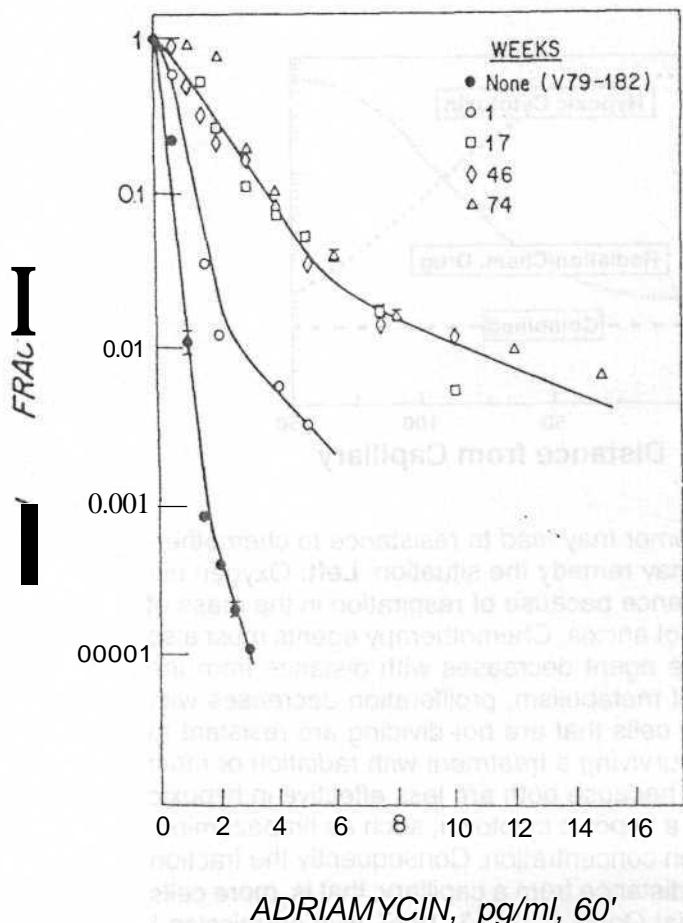
The biggest single problem in chemotherapy is drug resistance, which either may be evident from the outset or may develop during prolonged exposure to a cytostatic drug. Cells resistant to the drug take over, and the tumor as a whole becomes unresponsive. The development of resistance can be demonstrated readily for cells in culture. Figure 27.7 shows a substantial resistance to doxorubicin developing as cells are grown continuously in a low

concentration of the drug for a period of weeks.

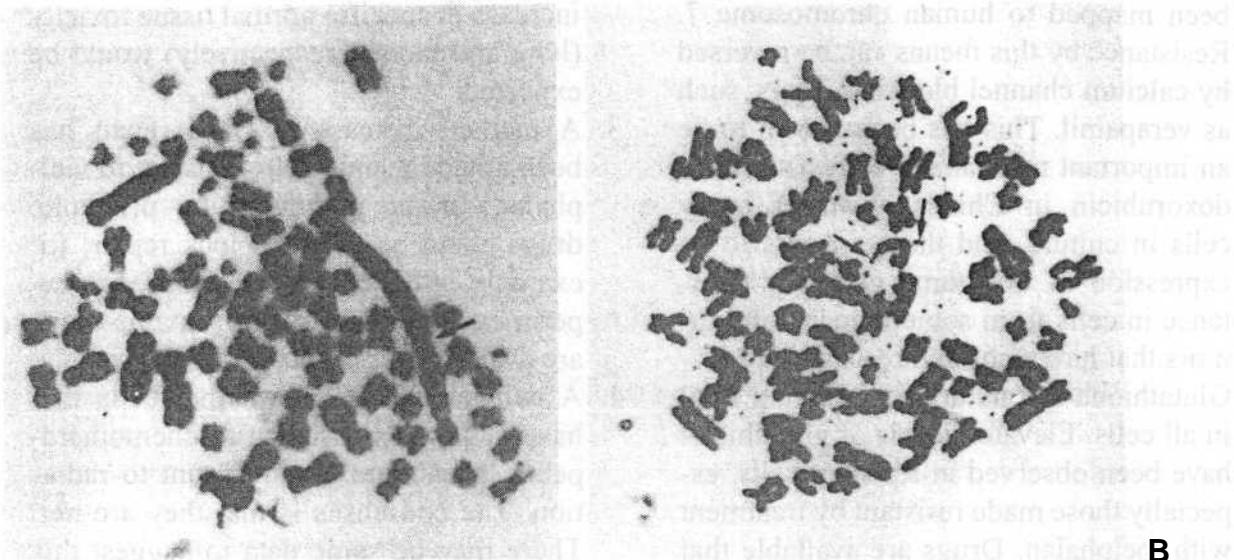
Underlying this problem of drug resistance are genetic changes that can sometimes be seen in chromosome preparations. Figure 27.8 shows two illustrations involving gene amplification or the presence of multiple minute chromosome fragments.

Drug resistance is an important factor that occurs readily—a phenomenon quite alien to the radiobiologist. Radiation-resistant cells can be produced and isolated, but it is a difficult and time-consuming process. For instance, cells continuously irradiated at low-dose rates occasionally do spawn radioresistant clones. By contrast, resistance to chemotherapeutic agents is acquired quickly, uniformly, and inevitably.

If a resistant clone can arise by a chance mutation of a gene responsible for one of the important steps in drug action, then the probability of it occurring would be expected to



**Figure 27.7.** Change in survival response to doxorubicin (Adriamycin) of Chinese hamster cells grown in culture and exposed continuously to a low concentration of the drug ( $0.05 \mu\text{m}/\text{ml}$ ) for prolonged periods of time, namely 1, 17, 46, or 74 weeks. The closed circles show the survival response for the parent cell line; a dramatic resistance to the drug develops by 17 to 74 weeks. (From Belli JA: Radiation response and Adriamycin resistance in mammalian cells in culture. Front Radiat Ther Oncol 13:9-20, 1979, with permission.)



**Figure 27.8.** Most forms of drug resistance probably have a genetic basis. A few extreme examples can be seen in chromosome changes. A: The arrow indicates an elongated chromosome, which on banding shows the features of an extended homogeneously staining region. This karyotype was observed in the human breast cancer cell line (MCF-7), which is resistant to methotrexate. (From Cowan KH, Goldsmith ME, Levine R, et al.: Dihydrofolate reductase gene amplification and possible rearrangement in estrogen-responsive methotrexate-resistant human breast cancer cells. *J Biol Chem* 257:15079-15086, 1982, with permission.) B: Small-cell lung carcinoma line derived from a patient treated with methotrexate. These cells are very resistant to the drug and contain numerous double-minute chromosomes. A pair is indicated by arrows. (From Curt GA, Carney DN, Cowan KH, et al.: Unstable methotrexate resistance in human small-cell carcinoma associated with double minute chromosomes. *N Engl J Med* 308:199-202, 1983, with permission.)

increase rapidly as the tumor increases in size. The average mutation rate for mammalian genes is about  $10^{-5}$  to  $10^{-6}$  per division, so that in a tumor containing  $10^{10}$  cells that go through many divisions the mutation is almost certain to occur, especially in the presence of a powerful mutagen, which most chemotherapeutic agents are.

The usual strategy to overcome the problem of induced resistance is to use a battery of different drugs, applied sequentially and cyclically, that produce their cytotoxicity by diverse mechanisms. By this strategy, cells that develop resistance to drug A are killed by drug B, and so on.

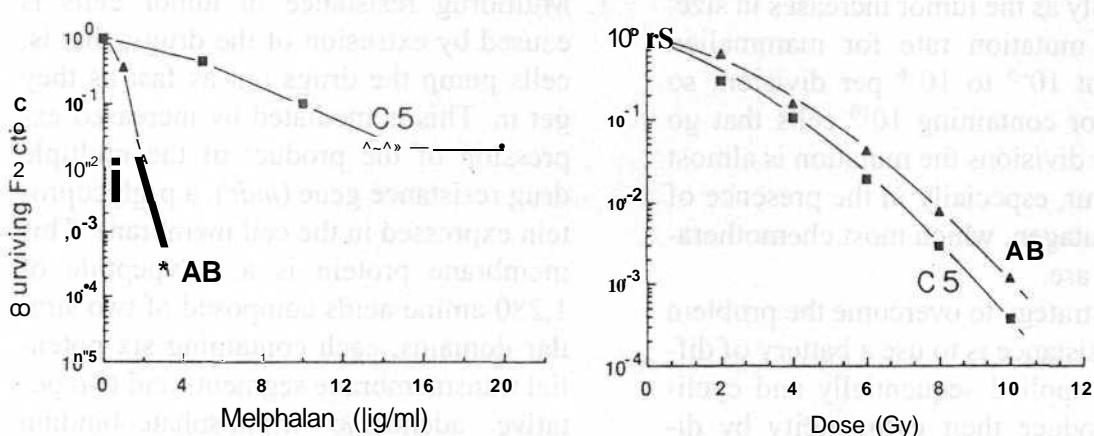
The bigger problem is pleiotropic resistance, the phenomenon by which the development of resistance to one drug results in cross-resistance to other drugs, even those with different mechanisms of action. There are four interesting points to be made:

1. Multidrug resistance in tumor cells is caused by extrusion of the drugs; that is, cells pump the drugs out as fast as they get in. This is mediated by increased expression of the product of the multiple drug resistance gene (*mdr*), a p-glycoprotein expressed in the cell membrane. This membrane protein is a polypeptide of 1,280 amino acids composed of two similar domains, each containing six potential transmembrane segments and two putative adenosine triphosphate-binding regions. Its structure is similar to that of various transporters of ions, amino acids, peptides, or proteins in bacterial, yeast, and animal cells. Indeed, it has been reported that the multidrug resistance gene in human tumor cells shows considerable homology to the gene in yeast, which extrudes an attractant that is important in the reproductive cycle. The *mdr* gene has

- been mapped to human chromosome 7. Resistance by this means can be reversed by calcium channel blocking drugs, such as verapamil. This has been shown to be an important mechanism of resistance to doxorubicin in Chinese hamster ovary cells in culture, and there appears to be expression of this same gene for resistance in cells from some human solid tumors that have acquired resistance.
2. Glutathione is a naturally occurring thiol in all cells. Elevated levels of glutathione have been observed in resistant cells, especially those made resistant by treatment with melphalan. Drugs are available that block the synthesis of glutathione and that can be used to lower the levels of this compound in tumors and normal tissues. The best known example is buthionine sulfoximine. Use of buthionine sulfoximine has been shown to reduce cross-resistance, particularly between melphalan and m-platinum in tumor-bearing mice. The use of buthionine sulfoximine would not be advisable in combination with doxorubicin or c/s-platinum, because an

increase in specific normal tissue toxicity (lung and kidney, respectively) would be expected.

3. A marked increase in DNA repair has been noted in some cells resistant to melphalan or c/s-platin. In principle, drugs could be used to block repair; for example, aphidicolin has been used in experimental systems, but no suitable drugs are available for clinical use.
4. A debatable issue is whether cells that have acquired resistance to chemotherapeutic agents are also resistant to radiation. The consensus is that they are not. There may be some data to suggest this from clinical experience, but the laboratory data show rather clearly that the acquiring of resistance to a drug does not necessarily result in radioresistance. This is illustrated in Figure 27.9, in which cells that have acquired extreme resistance to melphalan show a normal response to radiation. Radioresistance and chemoresistance may occur together, but radiation rarely induces chemoresistance and *vice versa*.



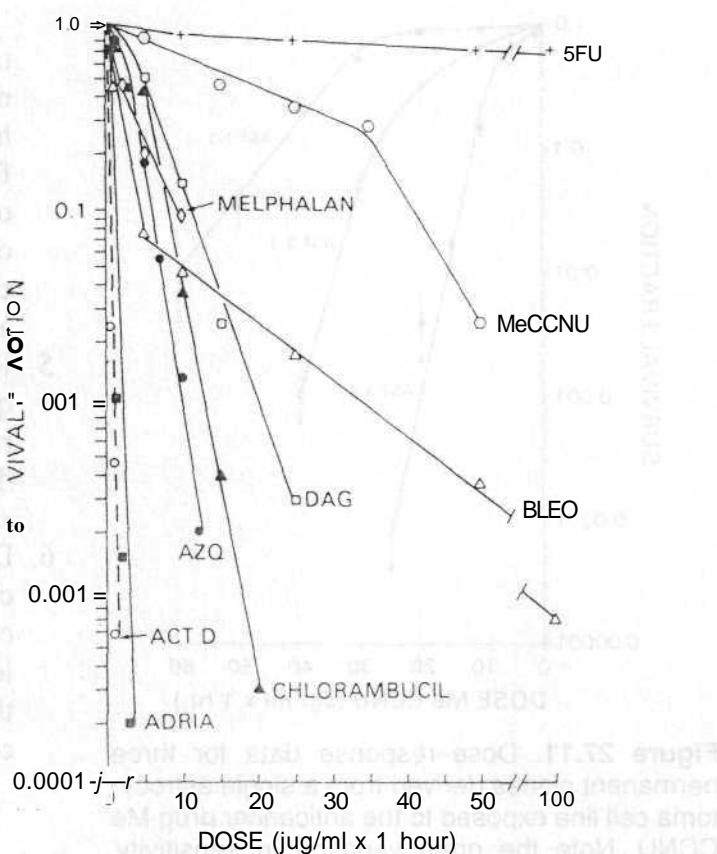
**Figure 27.9.** Chinese hamster pleiotropic multidrug-resistant cells are not necessarily resistant to radiation. The parallel ovary cell line is designated AB. The drug-resistant cell line C5 was isolated by Dr. Victor Ling by exposing the parental line to the mutagen ethyl methane sulfonate, after which surviving cells were grown for an extended period of time in increasing concentrations of colchicine. A clone was isolated that is resistant to colchicine and to a variety of chemotherapeutic agents. **Left:** C5 cells are resistant to melphalan, compared with the parental line (AB). They are also resistant to other agents, such as daunorubicin. **Right:** The radiation responses of the parental and the chemotherapy-resistant cell lines are virtually indistinguishable. (From Mitchell JB, Gamson J, Russo A, et al.: Chinese hamster pleiotropic multidrug resistant cells are not radioresistant. Natl Cancer Inst Monogr 6:187-191, 1988, with permission.)

The evolving story of drug resistance has an impact on the development and screening of new drugs. In the past, the initial screening for new agents consisted of fast-growing, highly drug-sensitive mouse tumors. Tests against specific patterns or types of drug resistance were not included. The screening systems, therefore, were weighted heavily in favor of producing more of the same types of drugs. This has changed, and the screening of new drugs for activity is performed using a battery of cells of human origin cultured *in vitro*.

### COMPARISON OF CHEMOTHERAPEUTIC AGENTS WITH RADIATION

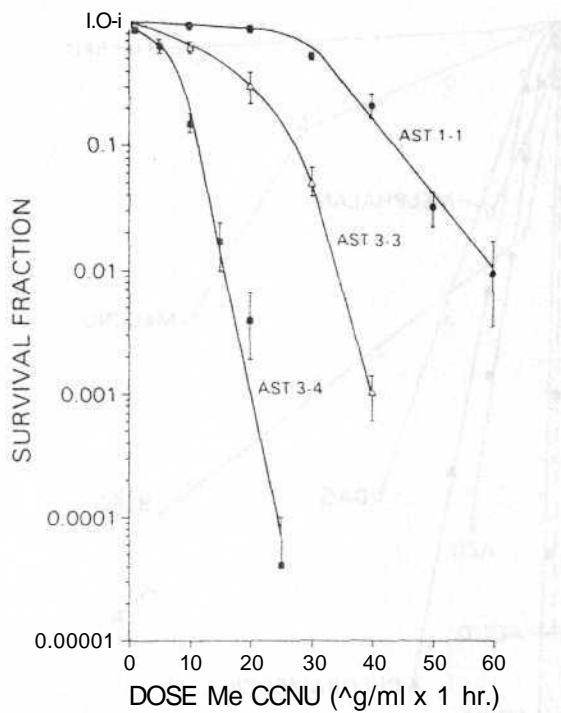
The title of this chapter includes the words, "from the perspective of the radiation biologist." This limited and specialized viewpoint must be kept in mind in what follows. A number of important differences are evident in the response of cells to chemotherapeutic agents *versus* ionizing radiation:

1. There is a much greater variation of sensitivity to chemotherapeutic agents than there is to radiation. In the case of x-rays the variation of  $D_0$  from the most sensitive to the most resistant known mammalian cells may be a factor of about 4. By contrast, the response of a variety of cell lines to a given chemotherapeutic agent may differ by orders of magnitude. A particular cell line may be exquisitely sensitive to one drug and extremely resistant to another. A different cell line may have a different order of sensitivity to various drugs, as well as a quite different absolute sensitivity. Different clones derived from a common stock may exhibit quite different sensitivity to a given agent. This variability is shown in Figure 27.10, which gives the response of one cell line to nine different cytotoxic agents, and in Figure 27.11, which shows the widely different response to CCNU of three clones derived from a common astrocytoma cell line.



**Figure 27.10.** Comparison of dose-response curves of a stomach cancer cell line in culture exposed for 1 hour to graded doses of nine anti-cancer drugs. There is a wide variation in sensitivity and in the shape of the various curves. (From Barranco SC, Townsend CM Jr, Quraishi MA, et al.: Heterogeneous responses of an *in vitro* model of human stomach cancer to anti-cancer drugs. *Invest New Drugs* 1(2): 117-127, 1983, with permission.)

2. The sensitivity of a given cell line to a given drug may be manipulated to a much greater extent than for radiation.
3. Repair of sublethal and potentially lethal damage is more variable and less predictable for drugs than for radiation.
4. The oxygen effect is more complex for drugs than for ionizing radiations. For radiation, the presence or absence of molecular oxygen has an important influence on the proportion of cells surviving a given dose of low linear energy transfer radiation, in which about two thirds of the damage is caused by indirect action (*i.e.*, mediated by free radicals). As the linear energy transfer



**Figure 27.11.** Dose-response data for three permanent clones derived from a single astrocytoma cell line exposed to the anticancer drug Me CCNU. Note the great variation in sensitivity. (From Rubin NH, Casantelli C, Maerk BG, Boerwinkle WR, Barranco SC: In vitro cellular characteristics and survival responses of human astrocytoma clones to chloroethyl-nitrosoureas and idanhydrogalactitol. Invest New Drugs 1: 129-137, 1983, with permission.)

of the radiation increases and the balance shifts from indirect to direct action, the importance of **oxygen** decreases. For very high linear energy transfer radiations (above about 200 keV/ $\mu\text{m}$ ) the biologic effect for a given dose is independent of the presence or absence of molecular oxygen. Under no circumstances is oxygen protective in the case of ionizing radiations.

For drugs **in** which the biologic effect involves free radicals, the presence or absence of oxygen is important in the same way as for low **linear** energy transfer ionizing radiations. The new factor **in** the case of drugs is that there is a whole class of antineoplastic agents that undergo bioreduction in the absence of oxygen, so that they are more effective in hypoxic cells. There is no parallel for ionizing radiations.

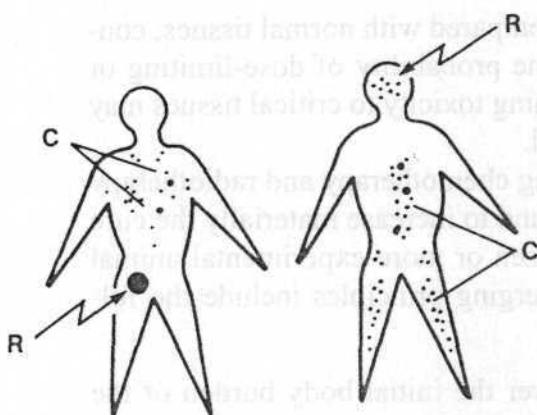
Other agents do not depend primarily on free radicals for their biologic effects, nor do they undergo bioreduction under hypoxic conditions; consequently, the effect of a given treatment is independent of the presence or absence of molecular oxygen, a property these agents have in common with very densely ionizing radiations.

5. Resistance to drugs develops more quickly and more regularly than it does to radiation. Acquired resistance to drugs does not necessarily involve resistance to x-rays as well.
6. Drug resistance may be caused by changes in **tfaoi** levels or by molecular changes observable at the chromosome level that result in the activation of a gene that functions to pump the drug out of the cells.

#### ADJUNCT USE OF CHEMOTHERAPEUTIC AGENTS WITH RADIATION

The initial rationale for the combination of radiation and chemotherapeutic agents was what usually is known as "spatial cooperation." Radiation may be more effective for controlling the localized primary tumor, because it can be aimed and large doses given (Fig. 27.12), but it is ineffective against disseminated disease. Chemotherapy, on the other hand, may be able to cope with micrometastases, whereas it could not control the larger primary tumor, in other situations (Fig. 27-12), chemotherapy is the primary treatment modality, and radiation is used only to treat "sanctuary" sites not reached by the drug.

Although spatial cooperation was the original rationale, it is no longer the only one. Radiation and chemotherapeutic agents are combined in an attempt to achieve better local control. A specific example is the integrated use of taxanes and x-rays for the treatment of breast cancer. This is based on laboratory experiments **in vitro**. The initial hypothesis for the mechanism of interaction is that **cell-cycle**

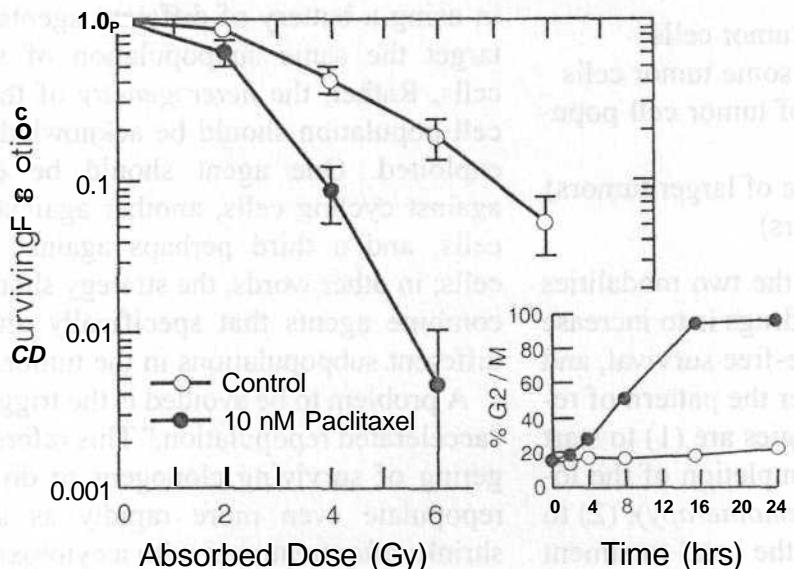


**Figure 27.12.** Spatial cooperation. In some instances radiation may be used to treat a large primary tumor with chemotherapy added to cope with systemic disease in the form of disseminated metastases. In other situations chemotherapy may be used as the primary treatment, with radiation added to treat "sanctuary" sites that the cytotoxic drug cannot reach.

alterations induced by paclitaxel leave cells in a state in which they are more sensitive to radiation. This is illustrated in Figure 27.13, which shows survival curves for astrocytoma cells of human origin, cultured *in vitro* and

exposed to graded doses of  $\gamma$ -rays, either alone or following a 24-hour treatment with 10 nm paclitaxel. This drug treatment alone kills about 85% of the cells and accumulates the survivors in the radiosensitive G<sub>2</sub>/M phase of the cell cycle (Fig. 27-13, inset). Inasmuch as the cell killing resulting from the combination of drug and radiation is greater than the sum of the two separately, the interaction may be described as "synergistic." For this to occur, the drug must accumulate some cells in G<sub>2</sub>/M, which is a radiosensitive phase of the cycle, without killing them. Although similar results have been obtained for several cell lines, there are a significant number of reports in the literature in which the interaction between radiation and paclitaxel was shown to be purely additive. Additivity is observed in any cell line in which the G<sub>2</sub>/M arrested cells are doomed to die as a consequence of the drug alone. Even an additive interaction, however, may be therapeutically useful. For example, in animal tumors *in vivo*, the paclitaxel-induced death of G<sub>2</sub>/M cells can lead to tumor shrinkage and reoxygenation, which result in

### Response of Human Astrocytoma Cells



**Figure 27.13.** Survival curves for astrocytoma cells of human origin exposed to graded doses of  $\gamma$ -rays alone and after a 24-hour treatment with a 10-nm concentration of paclitaxel. This drug concentration killed 95% of the cells and, as indicated by the inset, synchronized the survivors in the radiosensitive phase of the cell cycle. (Data from Tishler RB, Geard CR, Hall EJ, Schiff PB: Taxol sensitizes human astrocytoma cells to radiation. Cancer Res 52:3495-3497, 1992.)

an enhanced response to subsequent irradiation.

Although the initial rationale for the combination of taxanes and radiation was based on classic radiobiologic considerations regarding the cell-cycle dependence of radiosensitivity, there are other possible explanations for a synergistic effect if the two agents are used together. For example, the contribution of p53 to paclitaxel-dependent cytotoxicity is in marked contrast with its role for DNA-damaging agents such as radiation. Cells with a mutant p53 phenotype are more sensitive to paclitaxel treatment than are non-transformed or wild-type p53 cells. This finding offers an explanation for the effectiveness of paclitaxel as a cytotoxic agent alone, as well as for the efficacy of its combination with radiation. In this theory, paclitaxel and radiation act as non-cross-resistant agents; radiation is more effective against wild-type p53 cells in a tumor population, and paclitaxel is relatively more cytotoxic to cells with a mutant p53 phenotype.

In general, a therapeutic gain requires differential effects between tumor and normal tissue. One or more of the following tumor characteristics may be exploited to achieve this difference:

1. Genetic instability of tumor cells
2. Rapid proliferation of some tumor cells
3. Cell-age distribution of tumor cell populations
4. Hypoxia (characteristic of larger tumors)
5. pH (often low in tumors)

The goal of combining the two modalities of radiation and cytotoxic drugs is to increase local tumor control, relapse-free survival, and overall survival, and to alter the pattern of relapse. Three obvious strategies are (1) to start chemotherapy after the completion of the local treatment (*adjuvant chemotherapy*), (2) to start chemotherapy before the local treatment (*induction chemotherapy*), or (3) to give chemotherapy during local treatment (*concurrent chemotherapy*). Most cytotoxic agents do not provide enough differential sensitization

of tumors compared with normal tissues; consequently, the probability of dose-limiting or life-threatening toxicity to critical tissues may be increased.

Integrating chemotherapy and radiotherapy has been found to increase materially the cure rate of a dozen or more experimental animal tumors. Emerging principles include the following:

1. The lower the initial body burden of the tumor, the better.
2. A maximally effective drug regimen should begin as soon as possible after surgery, with maximum practicable doses.
3. Concurrent chemotherapy may lead to improved local control, but at the price of increased local toxicity.

Improving local control can improve overall survival rates and avoids uncontrolled local growth and the possible need for mutilating surgery. For example, induction chemotherapy prior to radiation therapy has a proven benefit in larynx preservation. For this reason alone aggressive use of combined modalities to improve local control is warranted.

It often has been said that "you can only kill the sensitive cells once." There is no point in using a battery of different agents that all target the same subpopulation of sensitive cells. Rather, the *heterogeneity* of the tumor cell population should be acknowledged and exploited. One agent should be effective against cycling cells, another against resting cells, and a third perhaps against hypoxic cells; in other words, the strategy should be to combine agents that specifically attack the different subpopulations in the tumor.

A problem to be avoided is the triggering of "accelerated repopulation." This refers to triggering of surviving clonogens to divide and repopulate even more rapidly as a tumor shrinks after treatment with a cytotoxic agent. It is a phenomenon described in Chapter 22. It may be one of the reasons why radiotherapy after induction chemotherapy has shown disappointing results.

## ASSAYS FOR SENSITIVITY OF INDIVIDUAL TUMORS

A great deal of effort has been expended to develop ways to assess which agents are likely to be effective for a particular tumor. The long-term goal would be to mimic the testing of a bacterial infection for sensitivity to a wide range of antibiotic drugs to select the one most suitable and effective.

One approach is to take biopsy specimens from a tumor in a patient, grow the cells *in vitro*, and subject the cells to a battery of chemotherapeutic agents in the petri dish. This approach has the advantages of not being too expensive to be practical and of providing answers quickly enough to influence the treatment and modify the protocol of the individual from whom the cells were taken. It suffers, of course, from the obvious disadvantages of focusing attention solely on the question of inherent cellular sensitivity and not addressing the questions of drug access, hypoxia, or any of the more complex factors involved as determinants of overall tumor response.

A different approach is to grow cells from human tumors as xenografts in immune-suppressed mice. This is a difficult and limited technique, which is beset with problems, some of which are discussed in Chapter 20. Human tumor cell xenografts, however, maintain many characteristics of the clinical response of the donor tumors. Indeed, there is a good correlation between clinical remission in donor patients and growth delay in xenografts established from transplanted cells. Establishing xenografts and performing the necessary growth-delay experiments is sufficiently slow and time consuming that the technique cannot be expected ever to provide realistic input into deciding treatment strategy in individual patients, although it can provide guidance on the sensitivity of broad categories of human tumors to a battery of chemotherapeutic agents.

More recent assays to predict responsiveness of individual tumors to radiation, chemotherapy, or a combination of both in-

clude the scoring of chromosome aberrations (specifically, micronuclei) in cells from treated tumors. This avoids many of the artifacts of the systems just discussed. Cells are not required to grow well in culture, only to be able to move into metaphase to express chromosome defects.

An additional approach that may be employed in the future to identify tumors resistant to a particular chemotherapeutic agent might be to test for the presence of specific genes that confer resistance.

## SECOND MALIGNANCIES

Late effects are the key to the acceptance of combined treatments. The induction of second malignancies is one of the unfortunate late effects of treatment **with** radiation or cytotoxic drugs. In a large series of 3,000 patients with Hodgkin's disease treated with a combination of radiotherapy and chemotherapy, 114 developed second malignancies. The greatest relative risk was leukemia, but the greatest in number were solid tumors.

Radiation is a relatively weak carcinogen; chemotherapeutic agents vary widely. There is a choice of many chemotherapeutic agents, and the variable potential for producing a second malignancy must be a factor influencing the choice of drug in patients who are likely to be long-term survivors.

Table 27.3 compares radiation, bleomycin, ultraviolet radiation, and benzopyrene in terms of the number of DNA lesions per cell necessary to kill 63% of the cell population, that is, to allow 37% to survive. Radiation is characterized by a relatively small number of double-strand breaks, only about 40 per cell on average, at a dose that allows 37% of the cells to survive. At the other extreme, ultraviolet light produces 1 million dimers, and benzopyrene produces 100,000 lesions for the same level of survival. These interesting figures show that radiation is a weak carcinogen, because it is efficient at killing cells; the same is true of bleomycin. By contrast, benzopyrene produces many more DNA lesions for a

TABLE 27.3. Lesions Produced for a Given Level of Cell Killing by Various Cytotoxic Agents

Agent	D <sub>37</sub>	DNA Lesion	Number of Lesions per Cell per D <sub>37</sub>
X-rays	100 rad	SSB	1,000
		DSB	40
Bleomycin	5.5 µg x 1 h	SSB	150
		DSB	30
Ultraviolet light	10 J/m <sup>2</sup>	TT dimer	1,000,000
		SSB	100
Benzopyrene	—	Adduct	100,000

SSB, single-strand DNA break; DSB, double-strand DNA break.

Courtesy of Dr. John Ward, University of California at San Francisco.

given level of cell killing and is therefore a powerful carcinogen; ultraviolet light is in the same category.

It is an interesting speculation, supported by these data, that the factor that determines whether an agent is a powerful or a weak carcinogen is the number of DNA lesions re-

quired, on average, to kill a cell. If the number is small, the agent is an efficient cytotoxic agent but is likely to be a weaker carcinogen. If the number is large, there will be many DNA lesions in cells that are not killed, and some of these lesions may involve the transformation to a neoplastic state.

### SUMMARY OF PERTINENT CONCLUSIONS

- Single agents have been used successfully to cure a few rapidly proliferating tumors.
- Combinations of drugs are used routinely for the treatment of a variety of malignancies.
- Most anticancer drugs work by affecting DNA synthesis or function.
- Inevitably, anticancer drugs are toxic to stem cells of the intestinal epithelium and hematopoietic stem cells, because they have a high growth fraction.
- Agents that are mainly effective during a particular phase of the cell cycle, such as the S phase or M phase, are said to be *cell-cycle specific*, or *phase-specific*.
- Agents whose action is independent of the position of the cell in the cycle are said to be *cell-cycle nonspecific*, or *phase-nonspecific*.
- Most commonly used chemotherapeutic agents fall into one of several classes:

*Alkylating agents*, which are highly active, with the ability to substitute alkyl groups for hydrogen atoms in DNA, include nitrogen mustard, cyclophosphamide, chlorambucil, melphalan, and the nitrosoureas (BCNU and CCNU).

*Antibiotics*, which bind to DNA and inhibit DNA and RNA synthesis, include dactinomycin, doxorubicin, daunorubicin, and bleomycin.

*Vinca alkaloids*, such as vincristine sulfate and vinblastine sulfate, cause mitotic arrest by binding to cellular microtubular proteins.

*Taxanes* are potent microtubule stabilizing agents and promoters of microtubule assembly and consequently block or prolong the transit time of cells through G<sub>1</sub> and M.

*Antimetabolites*, which are analogues of the normal metabolites required for cell function and replication, include methotrexate, 5-fluorouracil, cytarabine, and 5-azacytidine.

- Some important agents comprise a fourth "miscellaneous" group, including the platinum complexes and procarbazine.
- Dose-response relationships for many chemotherapeutic agents resemble those for radiation, with drug concentration replacing absorbed dose; that is, there is an initial shoul-

der followed by an exponential relationship between surviving fraction and dose. The exceptions are doxorubicin, bleomycin, dactinomycin, and taxanes, which have dose-response curves that are concave upward.

- At best, anticancer drugs kill cells by first-order kinetics; that is, a given dose kills a constant fraction of cells. Consequently, the chance of eradicating a cancer is greatest if the population size is small (*i.e.*, there is an inverse relationship between curability and tumor cell burden).
- Studies of sublethal damage and potentially lethal damage are more confusing and less clearcut for drugs than for radiation.
- Potentially lethal damage repair is a significant factor for bleomycin and doxorubicin, but sublethal damage repair is essentially absent. Neither potentially lethal nor sublethal damage repair is reported for the nitrosoureas.
- The oxygen effect is more complex for drugs than for radiation.
- Some drugs (*e.g.*, bleomycin) are more toxic to aerated than to hypoxic cells. For these drugs, free radicals are involved in the mechanism of cell killing, as is the case for x-rays.
- Some drugs (such as mitomycin C) are more toxic to hypoxic than to aerated cells, because they undergo bioreduction. This also applies to tirapazamine, as discussed in Chapter 25.
- Other drugs (including 5-fluorouracil, methotrexate, cis-platinum, and the nitrosoureas) appear to be equally cytotoxic to aerated and hypoxic cells.
- The effectiveness of chemotherapy agents decreases with distance from a capillary because the drug concentration falls off because of metabolism, and because cells are not proliferating because they are hypoxic.
- The addition of a hypoxic cytotoxin to other chemotherapy agents may alleviate this problem. As the effectiveness of conventional agents falls off with distance from a capillary, hypoxic cytotoxins become more effective, because they only function in low oxygen concentrations.
- Drug resistance is the biggest single problem in chemotherapy. For example, cells exposed continuously to low levels of doxorubicin become very resistant to subsequent treatments with this drug.
- The usual strategy to overcome resistance is to use a battery of drugs that produce cytotoxicity by diverse mechanisms.
- Pleiotropic resistance occurs if the development of resistance to one drug results in cross-resistance to other drugs with a different mechanism of action.
- Underlying acquired resistance are genetic changes.
- Resistance may be associated with the following:

Decreased drug accumulation and the expression of PI 70 glycoproteins in the cell membrane from gene amplification

Elevated levels of glutathione

Marked increase in DNA repair

- Radioresistance and chemoresistance may occur together, but radiation rarely induces chemoresistance, and *vice versa*.

- The adjunct use of chemotherapy with radiation may involve sequential or concurrent treatments.

- A therapeutic gain requires a differential between tumor and normal tissue. This may be achieved by exploiting one or more of the following tumor characteristics:

Genetic instability

Rapid proliferation

## Cell-age distribution

## Hypoxia

## pH

- Sensitive cells can be killed only once. Tumor heterogeneity should be exploited by using a combination of drugs effective against different cell subpopulations.
- Sensitivity of individual tumors to chemotherapeutic agents with or without radiation may be assessed by the following:

*In vitro* clonogenic assays

Xenografts in nude mice

Micronuclei in treated cells

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## Hyperthermia

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In Greek mythology the father of hyperthermia was Prometheus, a demigod who stole fire from Olympus and taught men to use it. For this act he was chained to a rock by Zeus, the chief of the gods, and a vulture fed daily on his liver.

The use of hyperthermia for the treatment of cancer is certainly not new. The very first medical text known today contains a case study describing a patient with a breast tumor treated with hyperthermia. The case is found in the Edwin Smith Surgical Papyrus, an Egyptian papyrus roll, which dates from more than 5,000 years ago. Later, heat was used in all cultures as one of the most prominent medical therapies for almost any disease, including cancer. Thus, Hippocrates (470-377 BC), in one of his aphorisms, states, "*Quae medicamenta non sanat, fentm sanat. Quae*

*ferum non sanat, ignis sanat. Quae vergo ignis non sanat, insanabilia repotari oportet*" ("Those who cannot be cured by medicine can be cured by surgery. Those who cannot be cured by surgery can be cured by fire [hyperthermia]. Those who cannot be cured by hyperthermia, they are indeed incurable.")

The attempt in modern times to exploit elevated temperatures to treat cancer has a history longer than the use of ionizing radiations. In 1866, 30 years before Röntgen discovered "a new kind of ray," the German physician W. Busch described a patient with a sarcoma in the face that disappeared after a prolonged infection with erysipelas, an infectious disease normally characterized by high fever. This and similar cases led the New York surgeon William B. Coley to believe that the bacteria causing erysipelas may be effective against

cancer. He extracted a toxin (Coley's toxin, or mixed bacterial toxin), with which he treated a number of patients.

Although it is difficult to evaluate the direct role of heat in this combined total-body hyperthermia and unspecific immunotherapy, the work by Coley initiated a number of other studies using local hyperthermia applied to patients and tumors in experimental animals. In 1898, Westermark, a Swedish gynecologist, published a paper describing a marked regression of large carcinomas of the uterine cervix after local hyperthermia, although the treatments involved were poorly controlled and the cases largely anecdotal. The use of hyperthermia, either alone or in combination with radiation, has been attempted at irregular intervals over the years but has never found a permanent place in the management of cancer. Historical reviews of the early clinical use of **hyperthermia** can be found in several excellent articles (eg., by Dewey and colleagues, 1977, and by Overgaard, 1984).

The interest in hyperthermia at the present time is based on documented clinical evidence of tumor regression as well as a biologic rationale and encouraging results from laboratory experiments.

### METHODS OF HEATING

The major problem in the development of clinical hyperthermia for cancer therapy has been the design of equipment to heat designated tumor volumes accurately and uniformly. Methods of local heating include (1) shortwave diathermy, (2) radiofrequency-induced currents, (3) microwaves, and (4) ultrasound.

Most of the work with cells cultured *in vitro*, as well as much of the early animal experimentation, involves heating by hot water baths. The simplest and **most** reliable way to heat a petri dish or a tumor transplanted into the leg of a mouse is to immerse it totally in a thermostatically controlled bath of water. Water temperature can be controlled within a fraction of a degree, and temperature measurement involves no problem, although even

in this simplest of situations the tumor may not be at the same temperature as the skin.

If localized hyperthermia is achieved by microwaves, radiofrequency-induced currents, or ultrasound, there are serious technical problems and limitations. In the case of microwaves, good localization can be achieved at shallow depths, but at greater tumor depths, even if the frequency is lowered to allow deeper penetration, the localization is much poorer and surface heating limits therapy. If ultrasound is used, the presence of bone or air cavities causes distortions of the heating pattern, but adequate penetration and good uniform temperature distributions can be achieved in soft tissues, particularly with ultrasound in focused arrays. In practice, then, tumors such as recurrent **chest-wall** nodules can be treated adequately with microwaves, and it should theoretically be possible to heat deep-seated tumors below<sup>7</sup> the diaphragm with focused ultrasound, regional microwave devices, or interstitial techniques.

In all cases, however, present methods of heating pose a problem, though significant progress has been made as a result of the clever application of focused arrays. For example, the use of multiphased arrays allows uniformity of temperature to the edge of the field. The picture is complicated, and it is unlikely that one simple answer to all the complex problems will be found. This is the area, then, in which engineering developments are needed and a breakthrough in basic science would be welcome.

One method of producing localized hyperthermia that suffers from fewer problems is the use of implanted microwave or radiofrequency sources. Good temperature distributions can be achieved and maintained if radiofrequency-induced currents or microwaves are applied to an array of wires actually implanted in the tumor and surrounding tissues. The "wires" used are frequently radioactive sources, so that heat and radiation can be combined. Alternatively, microwave coaxial antennae can be inserted into the catheters used to hold the radioactive iridium-92 wires, and deep tumor volumes heated from the in-

side out. Some of the most promising results have been obtained in this way for the small fraction of patients with cancer for whom an interstitial implant is practical.

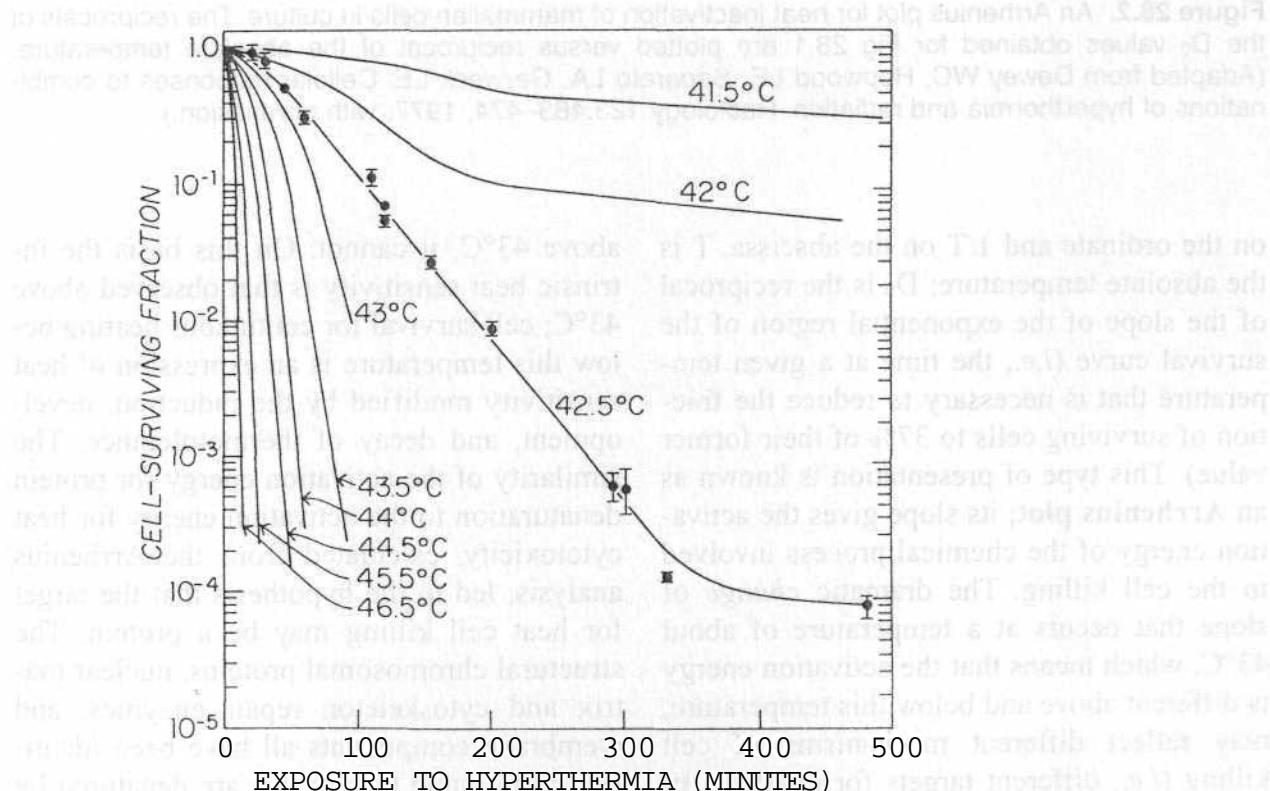
### CELLULAR RESPONSE TO HEAT

Heat kills cells in a predictable and repeatable way. Figure 28.1 shows a series of survival curves for cells exposed for various periods of time to a range of temperatures from 41.5°C to 46.5°C. The cell-survival curves for heat are similar in shape to those obtained for x-rays (*i.e.*, an initial shoulder followed by an exponential region), except that the time of exposure to the elevated temperature replaces the absorbed dose of x-rays. For lower temperatures the picture is complicated because the survival curves flatten out after a protracted exposure to **hyperthermia**, indicating the development of a resistance or tolerance to the elevated temperature. This is discussed sub-

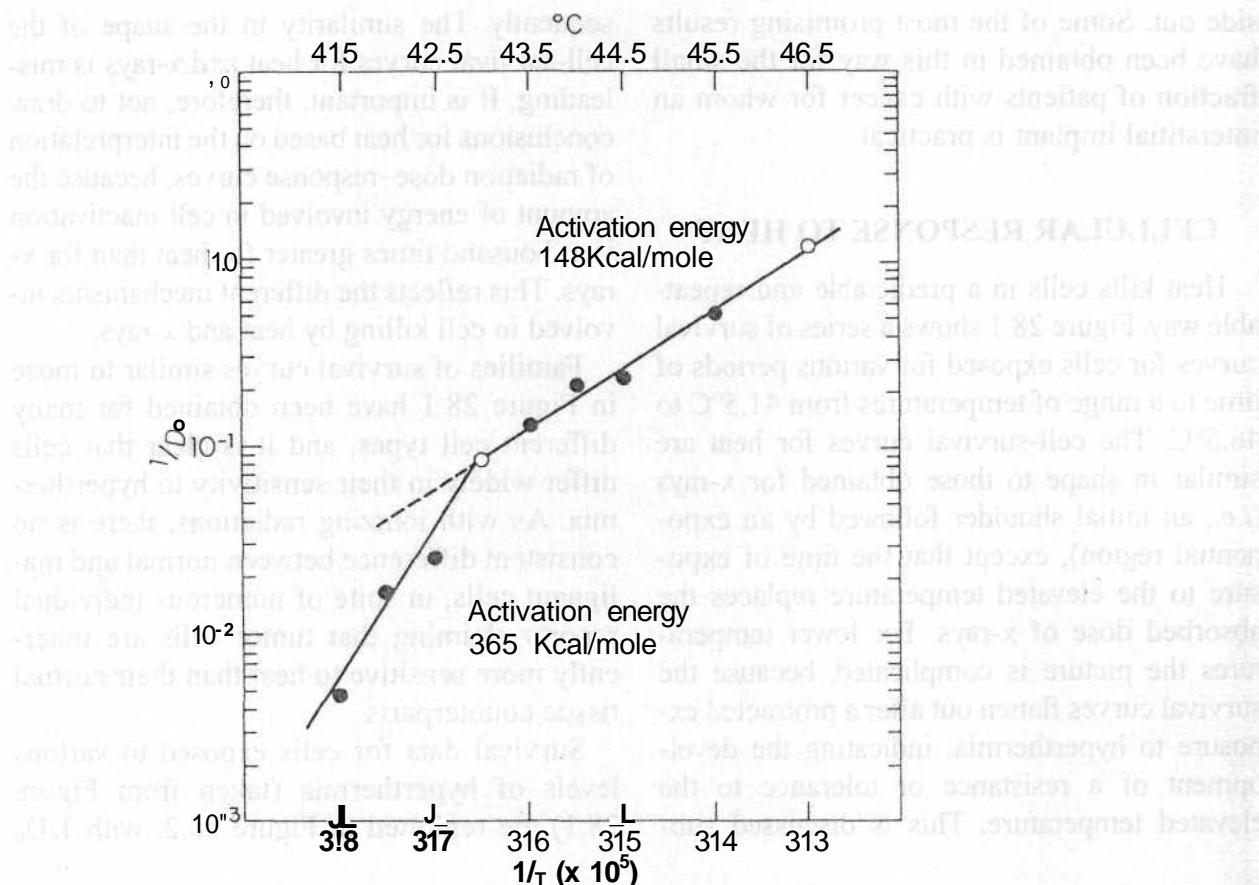
sequently. The similarity in the shape of the cell-survival curves for heat and x-rays is misleading. It is important, therefore, not to draw conclusions for heat based on the interpretation of radiation dose-response **curves**, because the amount of energy involved in cell inactivation is a thousand times greater for heat than for x-rays. This reflects the different mechanisms involved in cell killing by heat and x-rays.

Families of survival curves similar to those in Figure 28.1 have been obtained for many different cell types, and it is clear that cells differ widely in their sensitivity to hyperthermia. As with ionizing radiations, there is no consistent difference between normal and malignant cells, in spite of numerous individual reports claiming that tumor cells are inherently more sensitive to heat than their normal tissue counterparts.

Survival data for cells exposed to various levels of hyperthermia (taken from Figure 28.1) are replotted in Figure 28.2, with  $1/D_0$



**Figure 28.1.** Survival curves for mammalian cells in culture (Chinese hamster ovary line) heated at different temperatures for varying lengths of time. (Adapted from Dewey WC, Hopwood LE, Sapareto LA, Gerweck LE: Cellular responses to combinations of hyperthermia and radiation. Radiology 123: 463-474, 1977, with permission.)



**Figure 28.2.** An Arrhenius plot for heat inactivation of mammalian cells in culture. The reciprocals of the  $D_0$  values obtained for Fig 28.1 are plotted versus reciprocal of the absolute temperature. (Adapted from Dewey WC, Hopwood LE, Sapareto LA, Gerweck LE: Cellular responses to combinations of hyperthermia and radiation. Radiology 123:463-474, 1977, with permission.)

on the ordinate and  $1/T$  on the abscissa.  $T$  is the absolute temperature;  $D_0$  is the reciprocal of the slope of the exponential region of the survival curve (*i.e.*, the time at a given temperature that is necessary to reduce the fraction of surviving cells to 37% of their former value). This type of presentation is known as an Arrhenius plot; its slope gives the activation energy of the chemical process involved in the cell killing. The dramatic *change* of slope that occurs at a temperature of about 43°C, which means that the activation energy is different above and below this temperature, may reflect different mechanisms of cell killing (*i.e.*, different targets for cytotoxicity above and below 43°C). On the other hand, it may equally well be a manifestation of thermotolerance. Below 43°C, thermotolerance can develop gradually during the heating;

above 43°C, it cannot. On this basis the intrinsic heat sensitivity is that observed above 43°C; cell survival for continuous heating below this temperature is an expression of heat sensitivity modified by the induction, development, and decay of thermotolerance. The similarity of the activation energy for protein denaturation to the activation energy for heat cytotoxicity, calculated from the Arrhenius analysis, led to the hypothesis that the target for heat cell killing may be a protein. The structural chromosomal proteins, nuclear matrix and cytoskeleton repair enzymes, and membrane components all have been identified as possible targets that are denatured by hyperthermia.

The Arrhenius plot for a given cell line can be modified by a number of things. For example, altering the pH of the cells raises the

curves, and the break point occurs at a **higher** temperature.

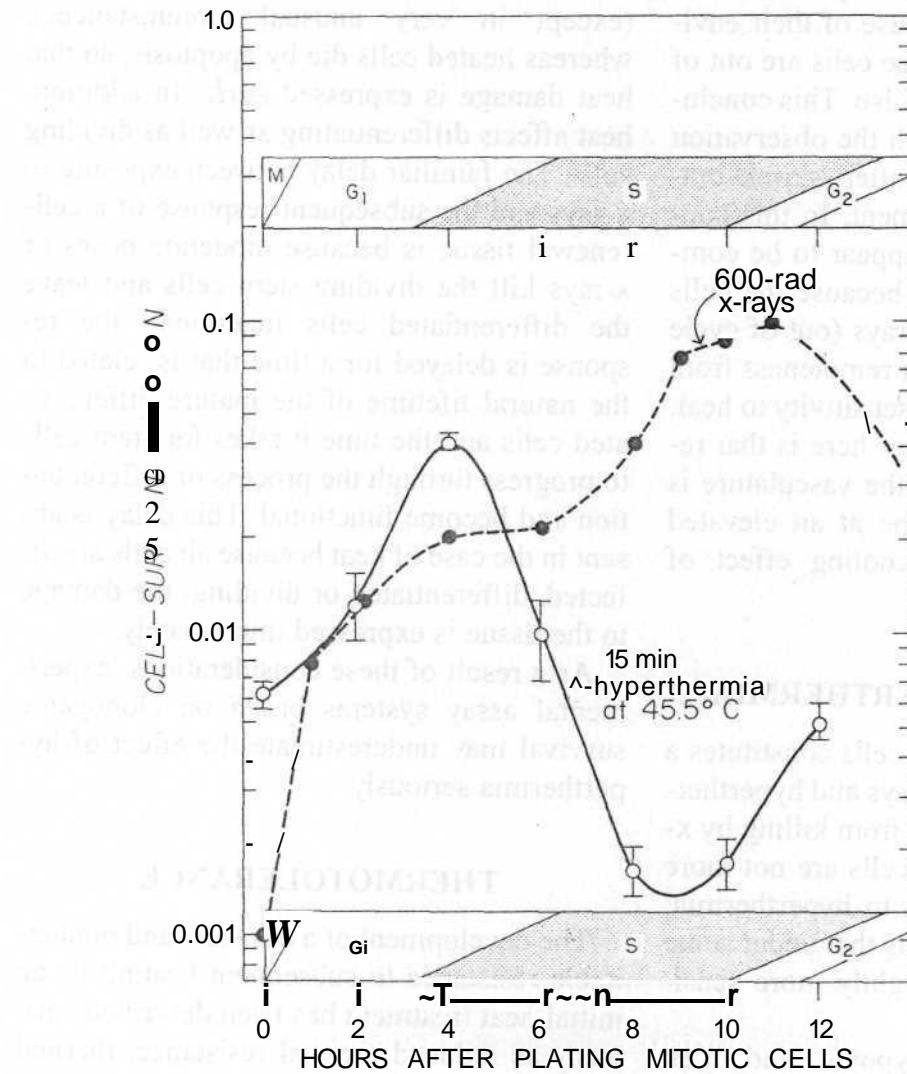
### SENSITIVITY TO HEAT AS A FUNCTION OF CELL AGE IN THE MITOTIC CYCLE

The age-response function for heat complements that for x-rays (Fig. 28.3). The phase of the cycle most *resistant* to x-rays, late in the DNA synthetic phase (late S), is most *sensitive* to hyperthermia treatment. On this basis, cycling tumor cells should be killed selec-

tively by hyperthermia compared with the slowly turning over normal tissues responsible for late effects that are in a Gi or Go state.

### EFFECT OF pH AND NUTRIENT DEFICIENCY ON SENSITIVITY TO HEAT

Cells at acid pH appear to be more sensitive to killing by heat. This is certainly true of cells treated with heat soon after their pH is altered artificially by adjusting the buffer. The pH dependence of cytotoxicity at elevated



**Figure 28.3.** Comparison of the fraction of cells surviving heat or x-irradiation delivered at various phases of the cell cycle. The heat treatment consisted of 15 minutes at  $45.5^{\circ}\text{C}$ , and the x-ray dose was 600 rad (6 Gy). (Adapted from Westra A, Dewey WC: Variation in sensitivity to heat shock during the cell cycle of Chinese hamster cells in vitro. Int J Radiat Biol 19:467-477, 1971, with permission.)

temperatures, however, is affected by pH history. Cells can adapt to pH changes and avoid the heat sensitivity shown for low pH.

Cells deficient in nutrients are certainly heat sensitive. This can be demonstrated with cells in culture in which sensitivity to heat increases progressively as cells have their energy supply compromised, either by depriving them of glucose or by the use of a drug that uncouples oxidative phosphorylation.

These conclusions about pH and nutrients, obtained under controlled conditions with cells in culture, led to the speculation that cells in tumors that are nutritionally deprived and at acid pH because of their location remote from a blood capillary may be particularly sensitive to heat. Because of their environment, it is likely that these cells are out of cycle and possibly hypoxic, also. This conclusion certainly correlates with the observation that large necrotic tumors often shrink dramatically after a heat treatment. In this context, also, heat and x-rays appear to be complementary in their action, because the cells that are most resistant to x-rays (out of cycle and hypoxic because of their remoteness from a capillary) show enhanced sensitivity to heat. A further complicating factor here is that regions of a tumor in which the vasculature is poorly developed tend to be at an elevated temperature because the cooling effect of blood flow is reduced.

### HYPOXIA AND HYPERHERMIA

The response of hypoxic cells constitutes a vital difference between x-rays and hyperthermia. Hypoxia protects cells from killing by x-rays. By contrast, hypoxic cells are not more resistant than aerobic cells to hyperthermia; indeed, the evidence suggests that under some conditions they may be slightly more sensitive.

Cells made acutely hypoxic and then treated with heat have a sensitivity similar to aerated cells. Cells subject to *chronic hypoxia* (*i.e.*, deprived of oxygen for prolonged periods) show a slightly enhanced sensitivity to heat. This increased sensitivity probably does

not result from hypoxia *per se* but may be a consequence of the lowered pH and the nutritional deficiency that cells suffer as a result of prolonged hypoxia. Of utmost importance in a practical situation is that hypoxic cells in tumors are often both nutrient-deficient and at a lowered pH.

### RESPONSE OF ORGANIZED TISSUES TO HEAT

Normal tissues respond to heat in a way that is substantially different from their more familiar response to x-rays. The principal difference is that after irradiation, cells die only in attempting the next or a subsequent mitosis (except in very unusual circumstances), whereas heated cells die by apoptosis, so that heat damage is expressed *early*. In addition, heat affects differentiating as well as dividing cells. The familiar delay between exposure to x-rays and the subsequent response of a cell-renewal tissue is because moderate doses of x-rays kill the dividing stem cells and leave the differentiated cells functional; the response is delayed for a time that is related to the natural lifetime of the mature differentiated cells and the time it takes for stem cells to progress through the process of differentiation and become functional. This delay is absent in the case of heat because all cells are affected, differentiated or dividing; the damage to the tissue is expressed immediately.

As a result of these considerations, experimental assay systems based on clonogenic survival may underestimate the effect of hyperthermia seriously.

### THERMOTOLERANCE

The development of a transient and nonheritable resistance to subsequent heating by an initial heat treatment has been described variously as induced thermal resistance, thermal tolerance, or, most commonly, thermotolerance. In 1975, Henle and Leeper and Gerner and Schneider independently showed that the resistance induced in cells by one heat exposure exceeded anything that could be ex-

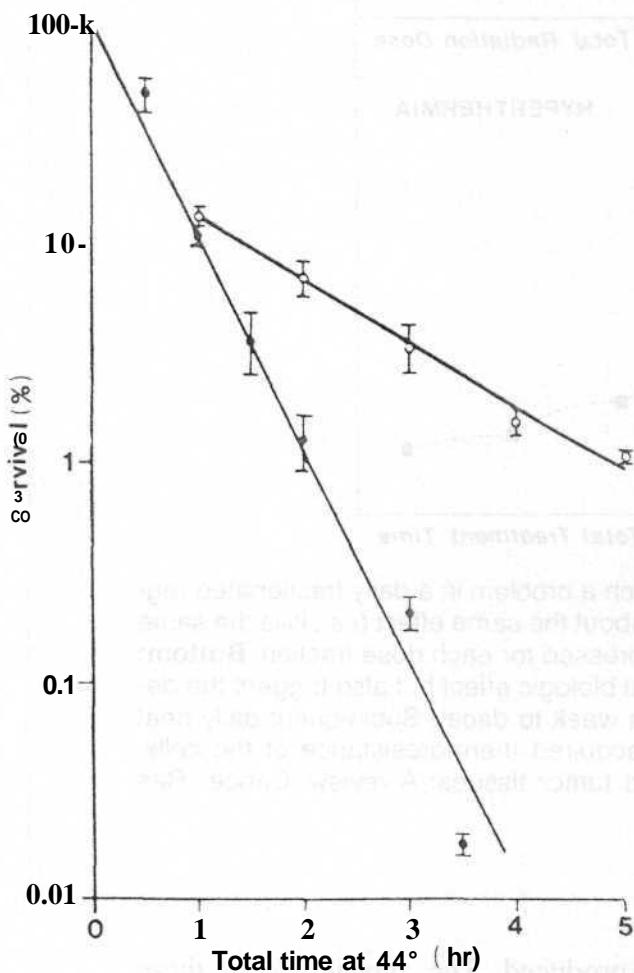
pected from repair of sublethal damage or progression into a resistance phase of the cycle. Figure 28.4 illustrates the phenomenon of thermotolerance. If heating at 44°C is interrupted after 1 hour and resumed some 2 hours later, the dose-response curve is much shallower (*i.e.*, the cells have become resistant) than if heating had been continued. Operationally, there are two ways in which heating can induce thermotolerance. First, at lower temperatures of around 39°C to 42°C, thermotolerance is induced during the heating pe-

riod after an exposure of 2 or 3 hours. This phenomenon is already apparent by the change of slope in the survival curves of Figure 28.1. Second, at temperatures above 43°C, thermotolerance cannot be produced during the heating, and it takes some time to develop after the heating has been stopped. It then decays slowly.

Thermotolerance is a substantial effect; the slope of a survival curve may be altered by a factor of 4 to 10, which translates into a difference in cell killing of several orders of magnitude. It is a factor to be reckoned with in fractionated hyperthermia. For cells in culture the time taken for cells that have become thermotolerant to revert to their normal sensitivity (*i.e.*, the decay of thermotolerance) may be as long as 160 hours. Groups led by Overgaard and by Urano have shown that thermotolerance can be induced in transplantable mouse tumors and that this thermotolerance also decays very slowly, requiring something on the order of 120 hours before the decay is complete.

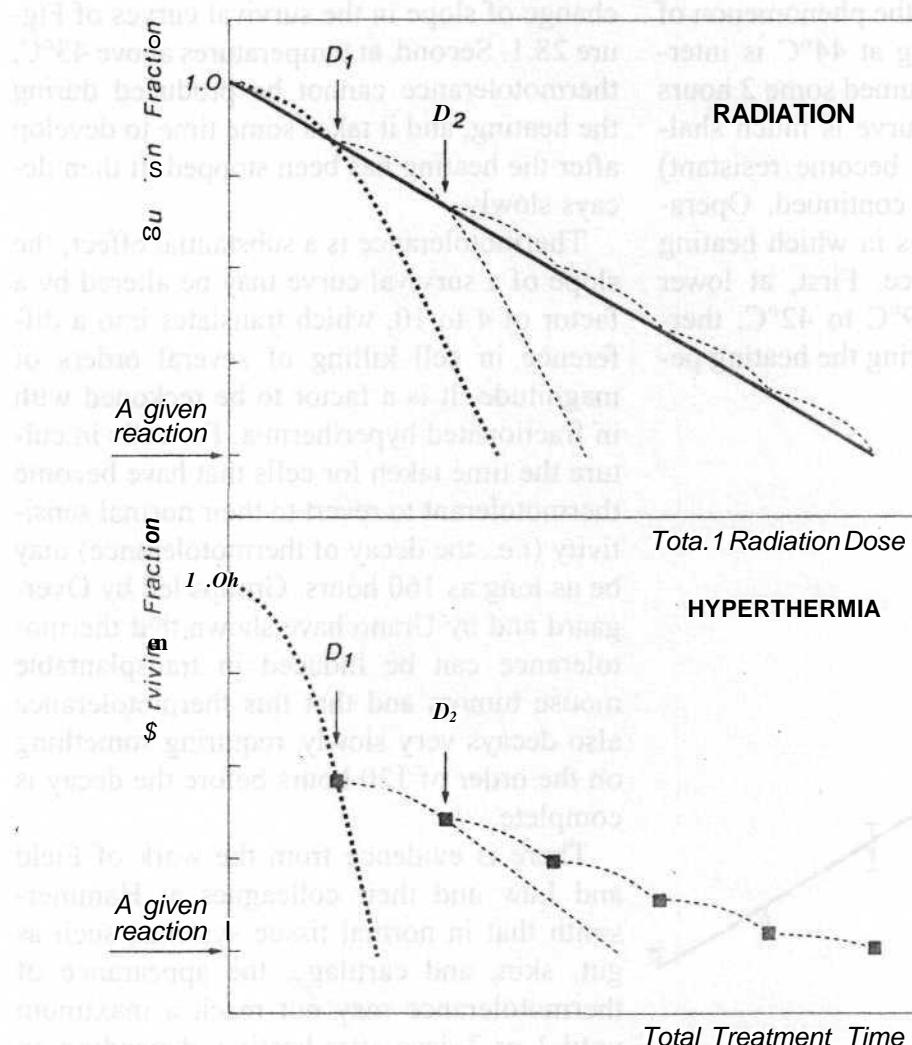
There is evidence from the work of Field and Law and their colleagues at Hammersmith that in normal tissue systems, such as gut, skin, and cartilage, the appearance of thermotolerance may not reach a maximum until 1 or 2 days after heating, depending on the initiating treatment. It may take as long as 1 or 2 weeks to decay completely.

Thermotolerance is a serious problem in the clinical use of hyperthermia. Figure 28.5 illustrates why by contrasting heat and radiation. The top graph shows the familiar pattern for a multifraction regimen of x-rays given in daily doses, in which the shoulder must be repeated each time and each dose produces about the same amount of cell killing. The bottom graph shows a strikingly different pattern for hyperthermia. The first heat dose kills a substantial fraction of cells, but subsequent daily treatments are comparatively ineffective because of the development of thermotolerance, which occurs a few hours after the first treatment and may take as much as a week to decay. Because of the problems and uncertainties involved, Field advises, "The best way to deal with ther-



**Figure 28.4.** Development of thermotolerance in HeLa cells. Closed circles indicate cell survival to single heat exposures; open circles indicate response of cells treated at 44°C for 1 hour, returned to 37°C for 2 hours, and given second graded doses of 44°C for graded times. (From Gerner EW, Schneider MJ: Induced thermal resistance in HeLa cells. Nature 256:500-502, 1975, with permission.)

### Cell Survival after Multiple Doses



**Figure 28.5.** Why the development of thermotolerance is such a problem in a daily fractionated regimen. **Top:** X-rays. Each dose in a fractionated regimen has about the same effect (i.e., kills the same proportion of cells). The shoulder of the curve must be reexpressed for each dose fraction. **Bottom:** Hyperthermia. The first heat treatment results in a substantial biologic effect but also triggers the development of thermotolerance, which may take as long as a week to decay. Subsequent daily heat treatments would be relatively ineffective because of the acquired thermoresistance of the cells. (From Urano M: Kinetics of thermotolerance in normal and tumor tissues: A review. *Cancer Res* 46:474-482, 1986, with permission.)

motolerance is to avoid it." This advice generally has been followed in the clinical use of hyperthermia, which imposes a limit of one or at most two heat treatments per week.

### HEAT-SHOCK PROTEINS

If cells are exposed to heat, proteins of a defined molecular weight (mainly 70 or 90

kd) are produced. The appearance of these heat-shock proteins tends to coincide with the development of thermotolerance and their disappearance with the decay of thermotolerance. Although the correlation is clear, it is not known if the heat-shock proteins are involved in the mechanism of the production of thermotolerance or an independent manifestation of the heat insult. In fact, although they

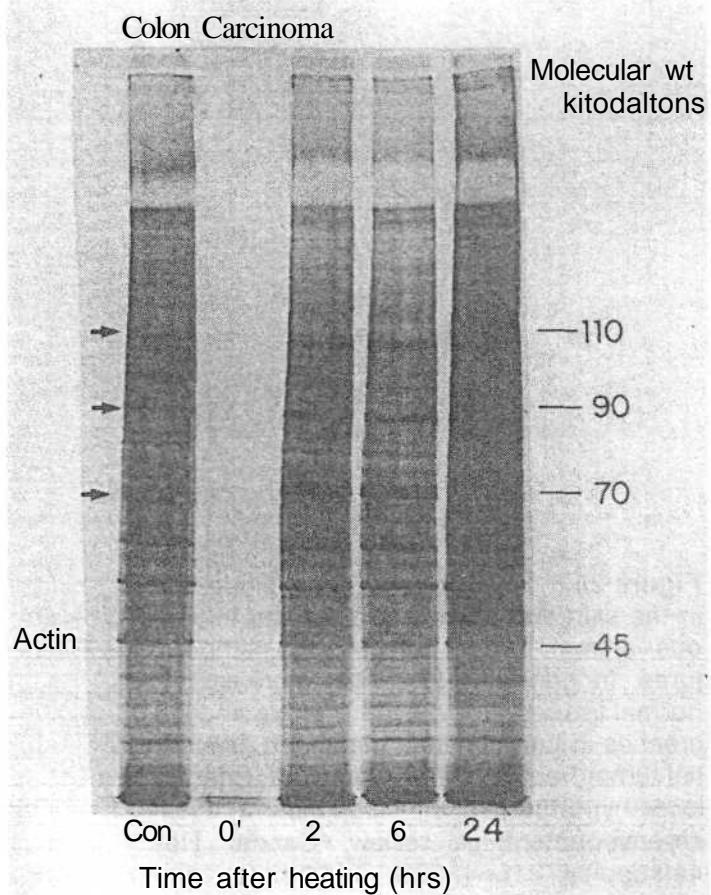
have been given the name *heat-shock proteins*, they are produced after treatment with other agents, including arsenite and ethanol. Proteins of roughly the same molecular weight are found in cells of many species and first were discovered, in fact, in the fruit fly *Drosophila*. They appear to be a ubiquitous cellular response to stress.

Heat-shock proteins are identified by gel electrophoresis, in which they show up as clearly defined bands of specific molecular weights. Methionine labeled with sulfur-75 is used to label the protein in the treated cells, which then are run on a polyacrylamide gel in an electric field. The proteins move a distance that depends on their molecular weight, with smaller proteins going farther. This separates out the various proteins in the cell (Fig. 28.6). After a treatment of 45°C for 20 minutes, proteins that are present only in small quantities before heat treatment are synthesized some

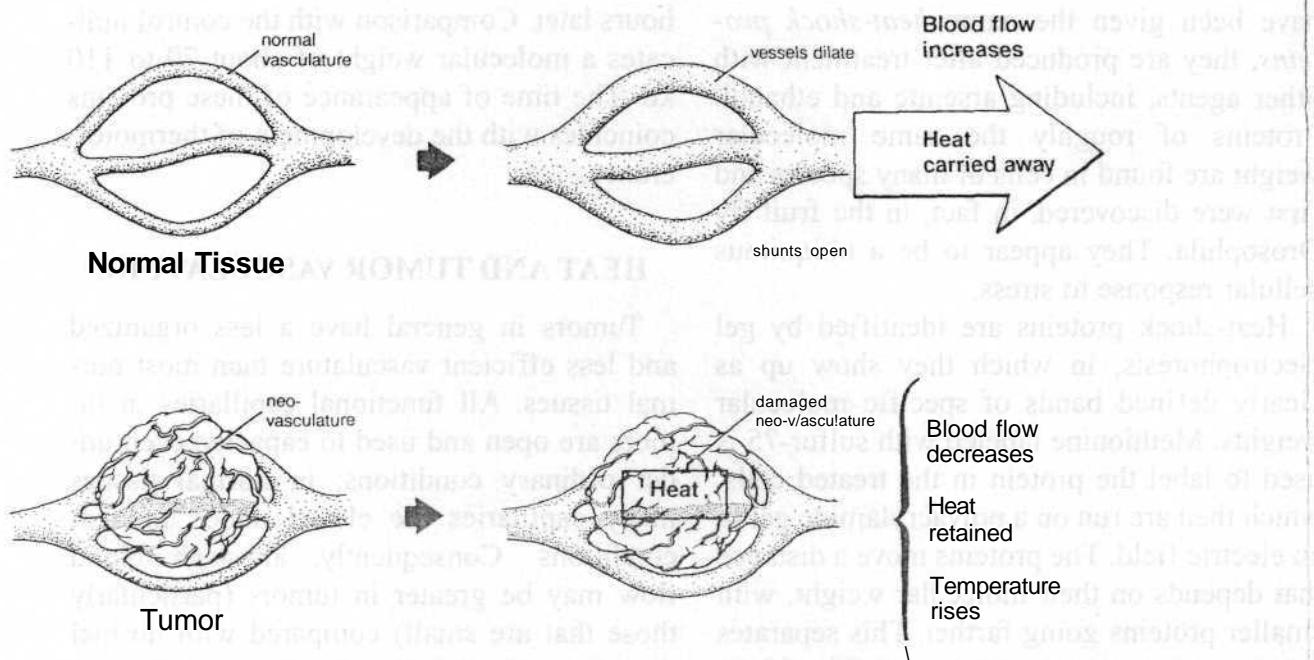
hours later. Comparison with the control indicates a molecular weight of about 70 to 110 kd. The time of appearance of these proteins coincides with the development of thermotolerance.

### HEAT AND TUMOR VASCULATURE

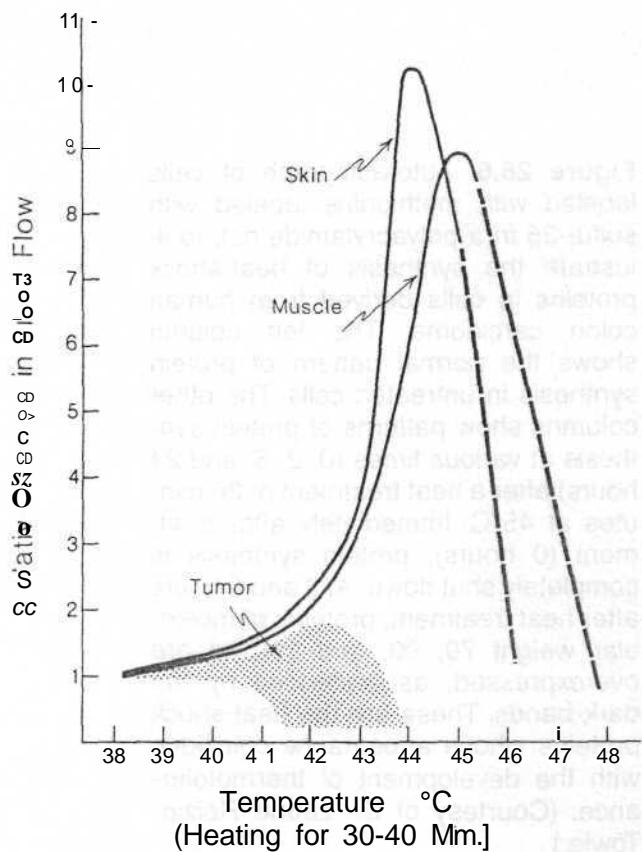
Tumors in general have a less organized and less efficient vasculature than most normal tissues. All functional capillaries in tumors are open and used to capacity, even under ordinary conditions; in normal tissues many capillaries are closed under ambient conditions. Consequently, although blood flow may be greater in tumors (particularly those that are small) compared with normal tissues at physiologic temperatures, the capacity of tumor blood flow to increase during heating appears to be rather limited in comparison with normal tissues. It is well docu-



**Figure 28.6.** Autoradiograph of cells labeled with methionine labeled with sulfur-35 in a polyacrylamide gel, to illustrate the synthesis of heat-shock proteins in cells derived from human colon carcinoma. The left column shows the normal pattern of protein synthesis in untreated cells. The other columns show patterns of protein synthesis at various times (0, 2, 6, and 24 hours) after a heat treatment of 20 minutes at 45°C. Immediately after treatment (0 hours), protein synthesis is completely shut down. At 2 and 6 hours after heat treatment, proteins of molecular weight 70, 90, and 110 kd are overexpressed, as evidenced by the dark bands. These are the heat-shock proteins whose appearance coincides with the development of thermotolerance. (Courtesy of Dr. Laurie Roizman-Towle.)



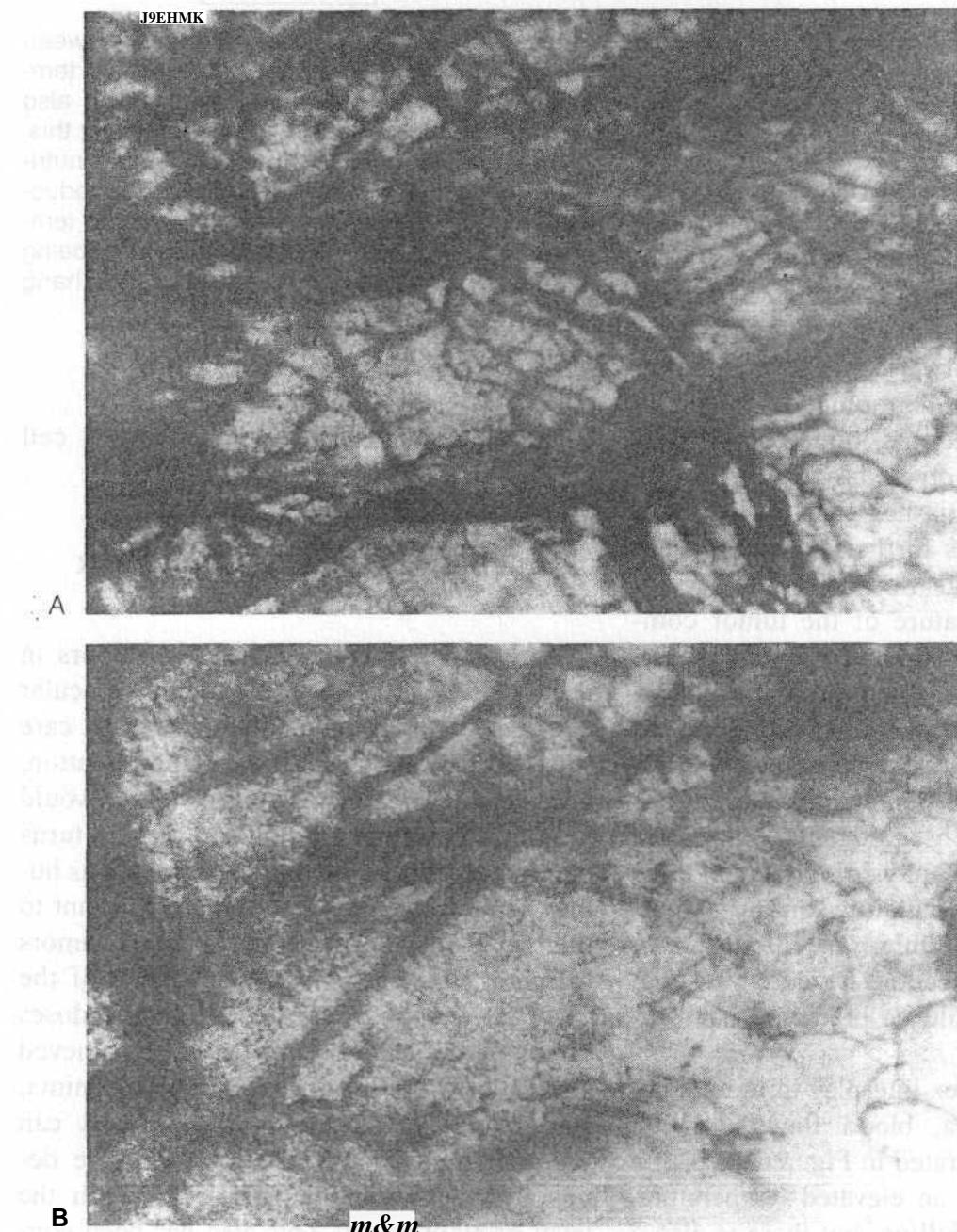
**Figure 28.7.** Possible mechanism explaining why tumors get hotter than surrounding normal tissues. Normal tissues have a relatively high ambient blood flow, which increases in response to thermal stress, thereby dissipating heat. Tumors, with relatively poor blood flow and unresponsive neovasculature, are incapable of augmenting flow (*i.e.*, shunting blood) and act as a heat reservoir. (Idea courtesy of Dr. F. K. Storm.)



**Figure 28.8.** Relative changes in blood flow in the skin and muscle of a rat and in various animal tumors at different temperatures. In general, blood flow increases in normal tissues during hyperthermia and decreases in tumors, often leading to differential tumor heating. (From Song CW: Effect of local hyperthermia on blood flow and microenvironment: A review. *Cancer Res* 44(suppl):4721S-4730S, 1984, with permission.)

mented that in normal tissues heat induces a prompt increase in blood flow accompanied by dilation of vessels and an increase in permeability of the vascular wall. As a result, heat dissipation by blood flow is slower in tumor than in normal tissues, and so it often is found that the temperature within a tumor is

higher than in the surrounding normal tissues. In a practical situation, therefore, the difference in intrinsic sensitivity between normal and malignant tissues becomes a moot point, because the tumor is often hotter anyway. A postulated mechanism for the selective solid tumor heating is shown in Figure 28.7. Lest it

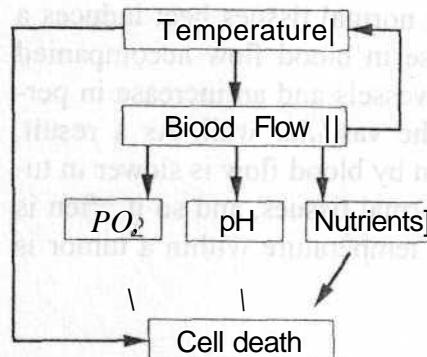


**Figure 28.9.** Hyperthermia-induced compression and occlusion of tumor vessels (x 10). The photographs are of a squamous cell carcinoma grown in a transparent cheek pouch chamber of a Syrian hamster. A: Vascular pattern at 34°C before heating. Note the prominent feeding arteriole. B: Occluded vascular network after 30 minutes of heating at 45°C. Blood flow to the area stopped because of marked constriction of the arteriole. Interstitial pressure then exceeded hydrostatic pressure to compress tumor vessels. (From Eddy HA: Radiology 137:515-521, 1980, with permission.)

should be thought that this is a function of the artificial "encapsulated" nature of most transplanted tumors in experimental animals, it should be noted here that differential heating of tumors relative to normal tissues frequently has been observed in humans in clinical trials of hyperthermia. It may be one of the reasons why excessive normal tissue damage has seldom been observed in clinical studies with heat.

There is, however, more to the story of heat and tumor vasculature. For reasons that are not fully understood, heat appears to preferentially damage the fragile vasculature of tumors; as a consequence, the heat-induced change in blood flow in at least transplantable tumors in animals is quite different from that in normal tissues. The relative change in blood flow in the skin and muscle of rats, as well as in various transplanted tumors, is summarized graphically in Figure 28.8. After hyperthermia, tumor blood flow in most cases goes *down*: blood flow in representative normal tissues goes *up* by a factor of 8 to 10. This further exacerbates the difference in blood flow and further increases the temperature of the tumor compared with the surrounding normal tissues. Eddy has studied the microcirculation of tumors after hyperthermia at 41 °C to 45°C. At the higher temperatures, particularly, compression, occlusion, hemorrhage, and stasis thrombosis were observed (Fig. 28.9). He concluded that pathophysiologic changes in the tumor microvasculature during hyperthermia can play an important role in tumor response and may account for cure rates that are better than would be predicted from direct cell killing.

There is a complex interplay in tumors between hyperthermia, blood flow, and cell killing; this is illustrated in Figure 28.10. If a tumor is heated to an elevated temperature, there is direct cell killing, but there is also a *reduction* in blood flow. This causes the tumor to get even hotter, because the reduced blood flow carries less heat away and at the same time the pH,  $pCO_2$ , and nutrient status of the



**Figure 28.10.** The complex interplay between hyperthermia and blood flow. An elevated temperature leads to direct cell killing, but it also causes a reduction in blood flow in tumors; this, in turn, causes changes in pH,  $pCO_2$ , and nutrients, leading to enhanced cell killing. The reduction in blood flow also further elevates the temperature in the tumor, because heat is not being carried away. (Based on the ideas of Dr. Chang Song.)

cells are affected, leading to enhanced cell killing.

## HYPERTHERMIA AND TUMOR OXYGENATION

Early studies with transplanted tumors in rodents showed that heat caused vascular damage. It was concluded therefore that care was needed in combining heat and radiation, because the oxygen status of the tumor would be compromised by heating. However, it turns out that tumor vasculature in spontaneous human tumors is considerably more resistant to thermal damage than in transplanted tumors in mice, and that in any case, much of the early work with mice utilized thermal doses far in excess of those that can be achieved clinically. It now is recognized from animal studies that mild hyperthermia actually can promote tumor reoxygenation, with the degree of reoxygenation correlating with the level of cytotoxicity. This also has been confirmed in a clinical study of patients with soft-tissue sarcomas. Brizel and colleagues showed that one heat treatment led to reoxygenation within 24 to 48 hours, whereas there

was no measurable reoxygenation during a week of standard radiation therapy.

### TEMPERATURE MEASUREMENT

The control and measurement of temperature in tissues, if heating is achieved by an external source, is not a trivial problem. If microwaves, short-wave diathermy, or radio-frequency-induced currents are used, accurate temperature measurements cannot be made with metal thermometers, such as thermocouples or thermistors, because of direct heating of the electrically conducting components and the perturbations of the electromagnetic field. These initial problems were solved largely by the development of nonmetallic, temperature-sensitive crystal and fiberoptic probes.

It is clearly desirable to replace invasive methods that measure temperature at a limited number of points with a noninvasive technique that gives an accurate picture of the temperature distribution. The most developed method of noninvasive thermometry is based on magnetic resonance imaging. A conventional magnetic resonance scanner, measuring the chemical shift of water, can yield a 0.5°C resolution in 0.02-cm<sup>3</sup> voxels in both normal and malignant tissues. This and similar ideas are likely to receive a lot of attention in the future.

### THERMAL DOSE

The temperature distribution in a tumor during hyperthermia treatment is almost always nonuniform. This nonuniformity stems from two spatially varying sources: power deposition and tumor blood perfusion, which carries the heat away.

The most common descriptors of the measured temperature distribution that are believed to correlate with biologic outcome are the minimum tumor temperature, because clonogens surviving in any region of lower temperature may constitute a focus for the regrowth of the tumor, and the temperature that

is exceeded by 90% of the measurements within the tumor.

As demonstrated by both *in vitro* and *in vivo* experiments, hyperthermia cytotoxicity is dependent on both temperature and time. The time-integrated temperature descriptor, namely **cumulative equivalent minutes at 43°C**, is perhaps the best concept of thermal dose.

A formal definition of thermal dose might be: *the time in minutes for which the tissue would have to be held at 43°C to suffer the same biologic damage as produced by the actual temperature, which may vary with time during a long exposure.*

Because in practice the tumor temperature may vary up and down during a typical treatment, some method is needed to convert the varying times at various temperatures into equivalent minutes at 43°C. Probably the best method proposed is to use the relationship between treatment time and temperature for a biologic isoeffect (the Arrhenius plot, shown for cells *in* culture in Figure 28.2). This has been confirmed in principle for a variety of normal tissues and tumors. A marked change of slope occurs somewhere between 42°C and 43°C. Above this transition temperature, the slope is consistent for a variety of cells and tissues. It generally is agreed that a 1°C rise of temperature is equivalent to a reduction of time by a factor of 2. Consequently, above this transition temperature,

$$\frac{t_2}{t_1} = 9^{T_1 - T_2}$$

in which  $t_1$  and  $t_2$  are the heating times at temperatures  $T_1$  and  $T_2$  to produce equal biologic effect.

For temperatures below the transition temperature, an increase in temperature by PC requires that time be decreased by a factor of 4 to 6:

$$\frac{t_2}{t_1} = (4 \text{ to } 6)^{T_1 - T_2}$$

The equivalent heating time at 43°C—the thermal dose—may be calculated from one or the other of these expressions or a combina-

tion of both. In principle, at least, the heat dose associated with a changing temperature may be calculated as the sum of equivalent heating times at 43°C for each temperature.

Although the concept of thermal dose is attractive, there are problems in its implementation:

- Nonuniformity of temperature occurs throughout the tumor.
- The concept relates only to cell killing by heat and does not include radiosensitization.
- It relates to one heat treatment, so it is not possible to add one treatment to the next given a few days later, because of the problem of thermotolerance.

### THE INTERACTION BETWEEN HEAT AND RADIATION

The biologic effect of a combination of heat and radiation may be a consequence of:

1. The independent but additive cytotoxic effects of the heat and radiation, with their complementary patterns of sensitivity through the cell cycle and the greater sensitivity to heat of nutritionally deprived cells at low pH
2. The interaction between heat and radiation, in the form of sensitization of the radiation cytotoxicity by heat, resulting from the inhibition of repair of radiation-induced damage

In a practical situation **in the clinic**, the interaction is most likely to be the independent and additive cytotoxic effects as described in the first point listed, because of the modest heat levels that can be achieved, and the time interval usually used between hyperthermia and radiation treatment. Of course, both may occur **simultaneously**, in which case the combination of hyperthermia and x-rays results in a greater cytotoxicity than can be accounted for by the addition of the cytotoxic effects of the agents alone; that is, the interaction between the two modalities is synergistic, or supra-additive. This interaction has been stud-

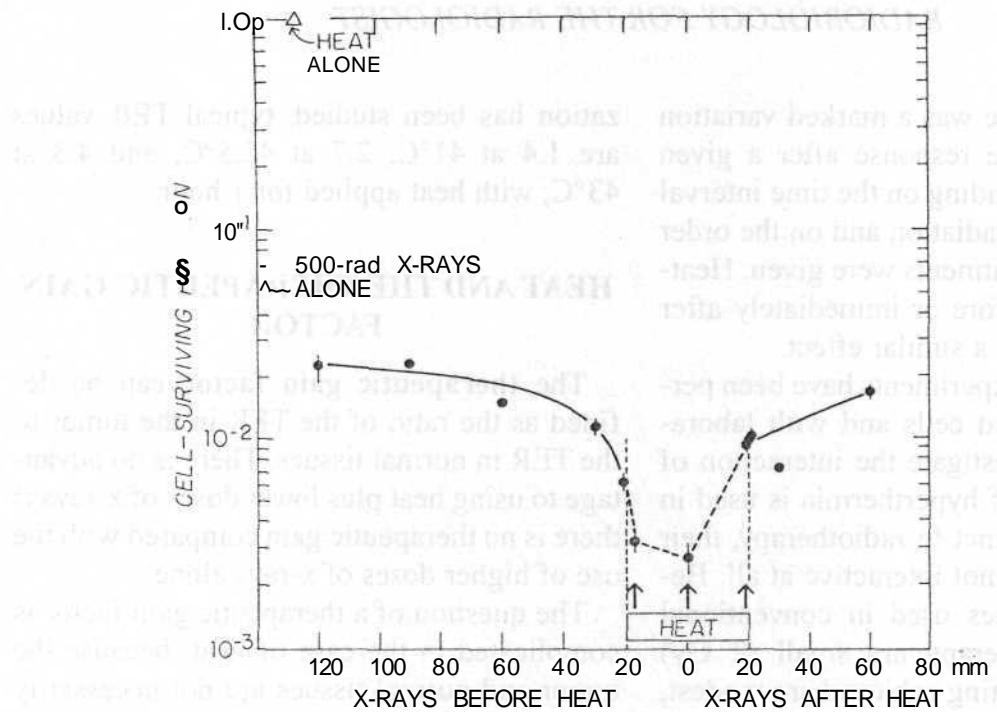
ied with cells in culture and with experimental animal systems.

In the case of cells cultured *in vitro*, the *shape* of the x-ray survival curve is changed by the addition of heat.

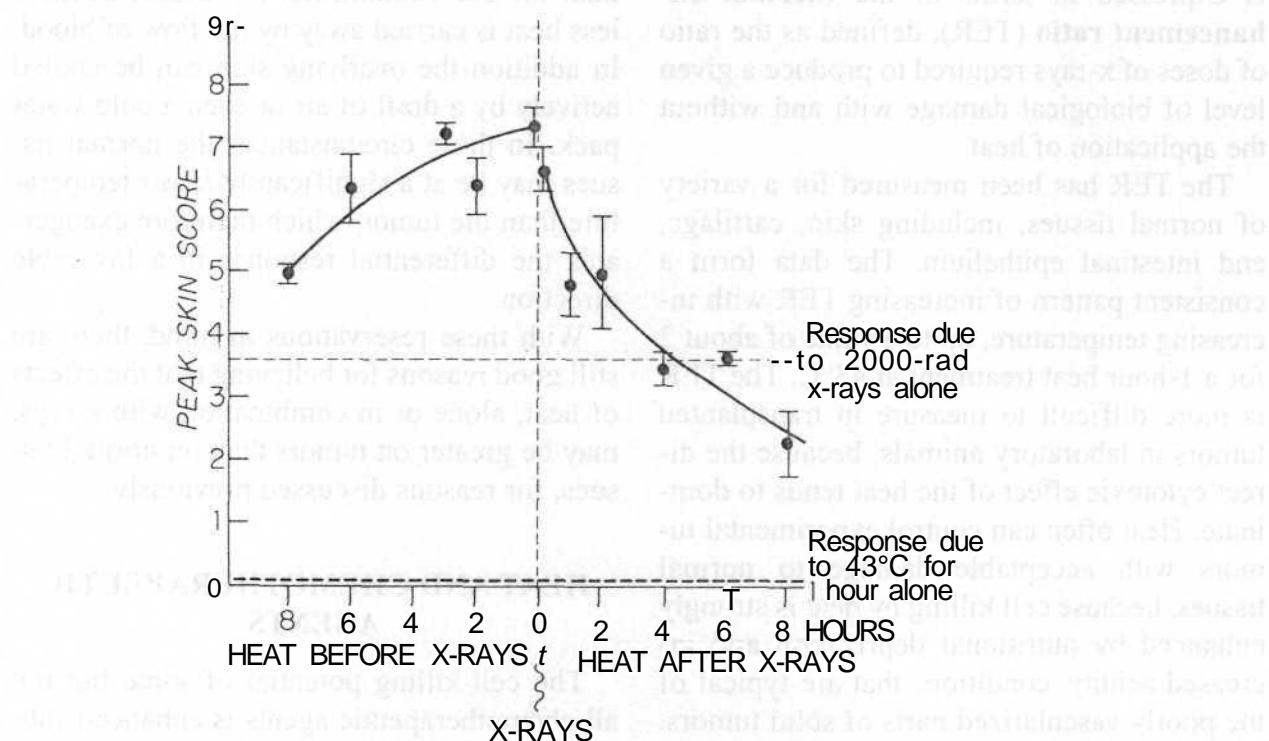
For *acute hyperthermia* (*i.e.*, brief exposures to temperatures of about 45°C, which lead to a substantial amount of cell killing) the principal effect of hyperthermia is a steepening (*i.e.*, reduced  $D_0$ ) of the x-ray survival curve. The change in the shoulder of the curve is minimal. On the other hand, if a more modest level of hyperthermia is used (4CM3°C), which involves little or no cell killing, the principal effect observed is a removal of the shoulder from the x-ray **survival** curve, and then heat treatment after irradiation is the more effective sequence. These differences between higher and lower temperatures, associated with the break in the Arrhenius plot at around 43 °C, may reflect different critical targets or simply be a consequence of the fact that thermotolerance can develop during the treatment at lower temperatures.

Heat inhibits the repair of radiation-induced single-strand breaks and radiation-induced chromosome aberrations. This inability to repair molecular damage translates into the inability to repair both sublethal damage and potentially lethal damage produced by radiation. Repair of sublethal damage does not occur if hyperthermia is applied during the interval between the two doses of x-rays.

If heat and radiation are combined, an important consideration is sequencing. Sequencing *in vitro* is discussed first. Figure 28.11 shows the fraction of cells that survived a combination of 5 Gy (500 rad) of x-rays and 40 minutes of heating at 42.5°C when the sequence of the two treatments and the time interval between them were **varied**. The heat treatment itself did not kill any cells, but it potentiated the effect of the x-rays, even if given an hour or more before or after the radiation. The greatest reduction in cell **survival** was caused by irradiation during heating. Comparable data for mouse skin are shown **in** Figure 28.12. Heating at 43°C for 1 hour was done before or after a single 20 Gy (2,000-rad)



**Figure 28.11.** Survival of Chinese hamster cells irradiated with 5 Gy (500 rad) of x-rays before, during, or after a heat treatment of 40 minutes at 42.5°C. No killing was observed with this heat treatment alone (*open triangle*). The effect of 5 Gy (500 rad) of x-rays alone is shown by the arrow. There is a clear interaction between heat and x-rays, with the maximum effect being produced if the x-rays are delivered midway through the heat treatment. (Adapted from Sarepato SA, Hopwood LE, Dewey WC: Combined effects of x-irradiation and hyperthermia on CHO cells for various temperatures and orders of application. *Radiat Res* 43:221-233, 1978, with permission.)



**Figure 28.12.** Response of mouse skin to the effect of combined heat and x-rays. The heat treatment was 43°C for 1 hour, and the x-ray dose was 20 Gy (2,000 rad). Heat was given before or after irradiation, and the time interval between the two was varied. (Adapted from Field SB, Hume S, Law MP, Morris C, Myers R: Presented at the International Symposium on Radiological Research Needed for the Improvement of Radiotherapy, Vienna, November 22-26, 1976, with permission.)

dose of x-rays. There was a marked variation in the normal tissue response after a given dose of x-rays, depending on the time interval between heat and irradiation and on the order in which the two treatments were given. Heating immediately before or immediately after irradiation produced a similar effect.

Although many experiments have been performed with cultured cells and with laboratory animals to investigate the interaction of heat and radiation, if hyperthermia is used in the clinic as an adjunct to radiotherapy, their effects are probably not interactive at all. Because the daily doses used in conventional fractionated radiotherapy are small (2 Gy) and the levels of heating achieved are modest, the cytotoxicity of the heat and radiation are more probably independent but additive.

### THERMAL ENHANCEMENT RATIO

In the case of either normal tissues or transplantable tumors in experimental animals, the extent of the interaction of heat and radiation is expressed in terms of the **thermal enhancement ratio** (TER), defined as the ratio of doses of x-rays required to produce a given level of biological damage with and without the application of heat.

The TER has been measured for a variety of normal tissues, including skin, cartilage, and intestinal epithelium. The data form a consistent pattern of increasing TER with increasing temperature, up to a value of about 2 for a 1-hour heat treatment at 43°C. The TER is more difficult to measure in transplanted tumors in laboratory animals, because the direct cytotoxic effect of the heat tends to dominate. Heat often can control experimental tumors with acceptable damage to normal tissues, because cell killing by heat is strongly enhanced by nutritional deprivation and increased acidity, conditions that are typical of the poorly vascularized parts of solid tumors. Thus a moderate heat treatment, which can be tolerated by well-vascularized normal tissues, destroys a large proportion of the cells of many solid tumors in experimental animals. In those cases in which thermal radiosensi-

zation has been studied, typical TER values are 1.4 at 41°C, 2.7 at 42.5°C, and 4.3 at 43°C, with heat applied for 1 hour.

### HEAT AND THE THERAPEUTIC GAIN FACTOR

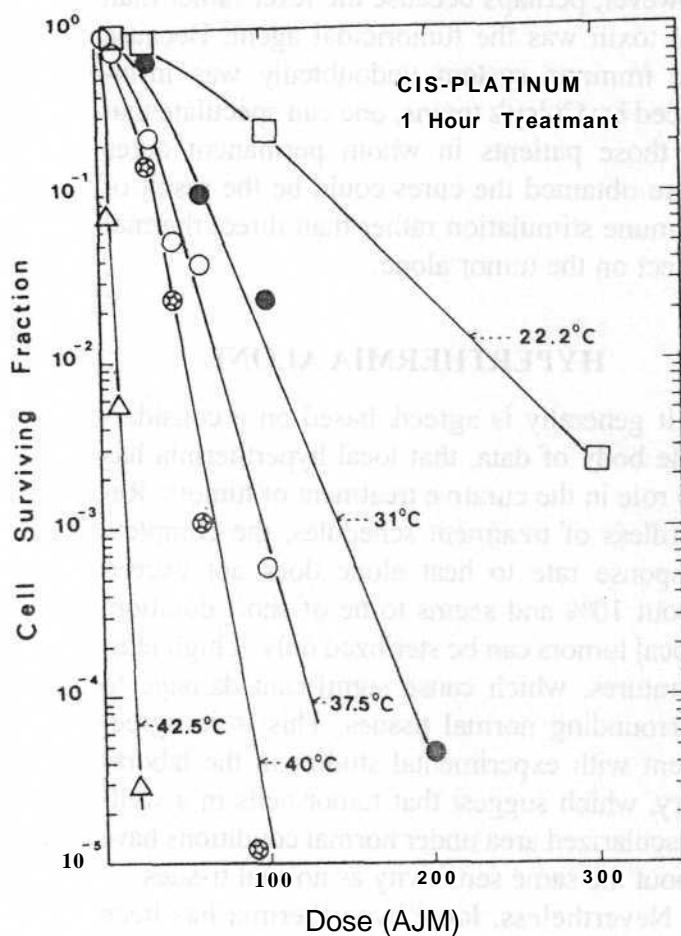
The **therapeutic gain factor** can be defined as the ratio of the TER in the tumor to the TER in normal tissues. There is no advantage to using heat plus lower doses of x-rays if there is no therapeutic gain compared with the use of higher doses of x-rays alone.

The question of a therapeutic gain factor is complicated in the case of heat, because the tumor and normal tissues are not necessarily at the same temperature. If the statement is made that heat preferentially damages tumor cells compared with normal tissue, it is implied that they both are at the same temperature. In a practical situation, however, this is not always the case. For example, if a poorly vascularized tumor is treated with microwaves, it may reach a *higher* temperature than the surrounding normal tissue, because less heat is carried away by the flow of blood. In addition the overlying skin can be cooled actively by a draft of air or even a cold **water pack**. In these circumstances the normal tissues may be at a significantly *lower* temperature than the tumor, which therefore exaggerates the differential response in a favorable direction.

With these reservations in mind, there are still good reasons for believing that the effects of heat, alone or in combination with x-rays, may be greater on tumors than on normal tissues, for reasons discussed previously.

### HEAT AND CHEMOTHERAPEUTIC AGENTS

The cell-killing potential of some but not all chemotherapeutic agents is enhanced substantially by a temperature elevation of even a few degrees. This is illustrated for cisplatin in Figure 28.13. There is also a striking synergism in cytotoxicity if hyperthermia (42–43°C) is combined with either bleomycin or



**Figure 28.13.** Effect of elevated temperatures on the cytotoxicity of cisplatin in V79 hamster cells *in vitro*. Cells were heated for 1 hour at the temperatures indicated. (From Roizin-Towle L, Hall EJ, Capuano L: Interaction of hyperthermia and cytotoxic agents. *Natl Cancer Inst Monogr* 61:149-151, 1982, with permission.)

doxorubicin. It is probable that at least part of the cell's sensitization to bleomycin at 43°C is related to the inhibition of a repair mechanism. No such explanation can account for the sensitization to doxorubicin, but it has been shown clearly that the drug gets into the cells more easily at 43 than at 37°C, possibly because of a change in the properties of the plasma membrane at the higher temperature. Once thermotolerance develops, however, less doxorubicin gets into the cell.

Whatever the mechanisms involved, the synergism between heat and drugs may prove very useful in the chemotherapy of solid tumors. The addition of local hyperthermia to a chemotherapy schedule would have the obvious advantage of "targeting" and localizing the principal effect of the drug, allowing greater tumor cell killing for a given systemic toxicity. This would help to overcome one of the principal problems and limitations of chemotherapy. It is surprising that more has

not been done in this area in view of the substantial potential benefits. Table 28.1 is a listing of drugs that are potentiated by heat and those that are not. There are several different mechanisms that may be involved, some of which are listed in Table 28.2.

**TABLE 28.1.** Interaction of Heat and Chemotherapeutic Agents

Effect	Drug
Potentiated by heat	Melphalan Cyclophosphamide BCNU Cis DDP Mitomycin C Bleomycin Vincristine Hydroxyurea Methotrexate Vinblastine Doxorubicin
Unaffected by heat	
Complex interaction	

From Kano E: Hyperthermia and drugs. In Overgaard J (ed): Hyperthermic Oncology, pp 277-282. London, Taylor & Francis, 1985, with permission.

TABLE 28.2. *Mechanisms of Interaction of Hyperthermia and Drugs*

Mechanism	Drug
Increased rate of alkylation	Thiotepa .. Nitrosoureas Mitomycin C Bleomycin Methylmethane sulphonate
Single-strand DNA breaks Inhibition of repair	Lonidamine Bleomycin Doxorubicin +/- Bleomycin - Melphalan +/- Thiotepa - Cyclophosphamide - Methyl-CCNU - Fluorouracil + Methotrexate +/- Cisplatin +/- Dactinomycin +/- Polyene antibiotics Local anesthetics Alcohols
Altered drug uptake <sup>a</sup>	Several Several
Common membrane target	
Energy metabolism Production of oxygen radicals	

Other mechanisms for the interaction of drugs and heat have been suggested, including increased ability of an agent to penetrate membranes and prolongation by drugs of the heat-sensitive cell-cycle phases.

<sup>a</sup>+, A reported increase from hyperthermia; -, a reported decrease.

Based on Dahl O: Hyperthermia and drugs. In Watmough DJ, Ross WM [eds]: Hyperthermia, Blackie, Glasgow, 1986, with permission.

### HUMAN APPLICATIONS

Hyperthermia has been widely used as a form of cancer therapy. Its use in the treatment of human tumors dates from early translations of Ramajama (2000 BC). In 1891, Coley noted that the regression of an inoperable "round cell sarcoma of the neck" was associated with a febrile bout of erysipelas. At this stage it was not clear whether the shrinkage of the tumor was caused by fever or a direct effect of the bacterial toxins. In 1953, Nauts, Fowler, and Bogatko repeated Coley's work, and 25 of their 30 selected patients with soft-tissue sarcoma, lymphosarcoma, or carcinoma of the cervix and breast were alive and disease-free at 10 years. The earlier results of Coley with the highly pyrogenic agents never were equaled with lesser agents or systemic hyperthermia,

however, perhaps because the fever rather than the toxin was the tumorcidal agent. Because the immune system undoubtedly was influenced by Coley's toxins, one can speculate that in those patients in whom permanent cures were obtained the cures could be the result of immune stimulation rather than direct thermal effect on the tumor alone.

### HYPERTHERMIA ALONE

It generally is agreed based on a considerable body of data, that local hyperthermia has no role in the curative treatment of tumors. Regardless of treatment schedules, the complete response rate to heat alone does not exceed about 10% and seems to be of short duration. Local tumors can be sterilized only at high temperatures, which cause significant damage to surrounding normal tissues. This is in agreement with experimental studies in the laboratory, which suggest that rumor cells in a well-vascularized area under normal conditions have about the same sensitivity as normal tissues.

Nevertheless, local hyperthermia has been shown to be useful in palliation, in the relief of pain. Treatment with heat alone, therefore, plays a limited role in cancer, and it is now usually recommended that it be performed only in patients who have limited life expectancies or if conventional therapy is contraindicated.

### HEAT PLUS RADIATION: CURRENT STATUS OF CLINICAL STUDIES

Hyperthermia has been used extensively as an adjuvant to radiation in the treatment of local and regional cancer, and this is the area in which it appears to offer the biggest advantage. The protocol that has emerged from the clinic is of the sequential application of radiation and heat, with one or two applications of heat interspersed with a normal multifraction course of radiotherapy.

The radiosensitizing effect of heat is most evident if the heat and radiation are applied simultaneously, but the effect is generally of the same magnitude in both tumors and normal

tissues and does not improve the therapeutic ratio unless the tumor is heated to a higher temperature than the normal tissue. On the other hand, the hyperthermic cytotoxic mechanism predominates if heat follows radiation in a sequential procedure. The heat-sensitive cells are those found in a nutritionally deprived, chronically hypoxic and acidic environment, conditions that are found in tumors but not generally seen in normal tissues. In addition, as a result of heat killing of the hypoxic radioresistant tumor cells, the actual radiation dose necessary to effectively control the tumor should be reduced. Thus, not only do we see a preferential enhancement occurring in tumors, but the overall radiation damage to normal tissues also is decreased, because the radiation dose is decreased. Consequently, a therapeutic gain should result.

A sequential administration of radiation and heat has several other advantages over a simultaneous treatment. The aim of giving heat is to improve the already existing radiotherapy treatment. If heat is combined with radiation in a simultaneous protocol, it has to be given with each radiation fraction. Thermotolerance then becomes a problem. This can be overcome by increasing the fraction interval, but that would be expected to reduce the effectiveness of the radiation alone. With sequential heat and radiation treatment the heat can be administered once or twice, without interfering with the normal radiotherapy protocol. Last but not least, the sequential application of the two modalities is much more practical; to heat and irradiate simultaneously would be very difficult indeed.

There is abundant evidence from phase I and II trials that heat may enhance radiotherapy to a significant degree; these data were chronicled in some detail in previous editions of this book. In more recent years, a number of randomized phase III multicenter trials have been conducted that must replace the more extensive, but less convincing, data from the past. The results of these trials are summarized in Table 28.3. Some show a benefit for the addition of hyperthermia as an adjuvant to radiation therapy, and some do not. The trials involving advanced breast cancer

and neck nodes with full-dose radiation therapy do not show any benefit from the use of hyperthermia. It may be that heating techniques were inadequate in these sites. Three trials show a clear and significant advantage for the addition of hyperthermia to standard radiation therapy. These may be summarized as follows.

### Superficial Localized Breast Cancer

An international collaborative hyperthermia group combined the results of five randomized controlled clinical trials conducted in the United Kingdom, Europe, and Canada, in which external-beam radiation therapy alone was compared with radiation therapy plus hyperthermia. Hyperthermia was induced using various externally applied electromagnetic applicators, aiming at a tumor temperature of 42.5 or 43°C. In fact, the minimum temperature rarely exceeded 41°C, because of difficulties in heating. Not all trials demonstrated an advantage for the combined treatment, but the pooled data showed a significant improvement in complete tumor response. The greatest effect was seen in patients with recurrent lesions in previously treated areas, in whom further irradiation was limited to low doses.

### Recurrent or Metastatic Malignant Melanoma

Within the framework of the European Society for Hyperthermic Oncology, a multicenter randomized trial show improved local control at 2 years and survival at 5 years for patients with recurrent malignant melanoma. The patients received three fractions of 8 or 9 Gy (800 or 900 rad) within a period of 8 days, either alone or combined with hyperthermia aiming at 43°C for 60 minutes.

### HYPERTHERMIA AND IMPLANTS

The dose-rate effect was described in detail in Chapter 5. In short, if x- or y-rays are delivered at low dose rates, the biologic effectiveness of a given dose is reduced because of the repair of sublethal damage that takes place dur-

TABLE 28.3. Results of Six Randomized Clinical Trials Comparing the Response of Thermoradiotherapy Versus Radiotherapy Alone

Site	Number of Lesions	Response					
		Complete Response, %		Local Control, 2 y (%)		Survival, 5 y (actuarial), %	
		HT+RT	RT	HT+RT	RT	HT+RT	RT
Head and Neck <sup>a</sup> (Stage III & IV)	65	46	12	25	8		
Head and Neck <sup>a</sup> (Stage IV nodes)	44	83	41	69	24	53	0
Melanoma <sup>c</sup>	134	62	32	46	28		
Breast <sup>d</sup> (Primary and recurrent)	396	59	41	45	29		
Breast <sup>d</sup> (Recurrent, previously irradiated)	210	57	31				
Glioma <sup>e</sup> (Interstitial HT+RT and RT)	64			Survival = 91 wk	Survival = 76 wk; P = 0.014		
Pelvic Tumors <sup>h</sup>			P = 0.005			3 yr Survival	P=0.014
Overall	360	56	39	p=0.013		32	24
Cervix <sup>1</sup> (68 Gy)	115	83	57			52	27
Bladder <sup>f</sup> (66 Gy)	102	74	51			NS	
Rectum <sup>g</sup> (56 Gy)	143	21	15			NS	

All differences significant to  $P < 0.05$ .

HT + RT, thermoradiotherapy; RT, radiotherapy.

<sup>a</sup>Datta NR, Bose AK, Kapoor HK, Gupta S. Head and neck cancers: Results of thermoradiotherapy versus radiotherapy. Int J Hypertherm 6:479-486, 1990.

<sup>b</sup>Valdagni R, Amichetti M: Report of long-term follow-up in a randomized trial comparing radiation therapy and radiation therapy plus hyperthermia to metastatic lymph nodes in stage IV head and neck patients. Int J Radiat Oncol Biol Phys 28:163-169, 1994.

<sup>c</sup>Overgaard J, Gonzalez Gonzalez D, Hulshof MCCM, et al.: Randomized trial of hyperthermia as adjuvant to radiotherapy for recurrent or metastatic malignant melanoma. Lancet 345:540-543, 1995.

<sup>d</sup>Vernon CC, Hand JW, Field SB, et al.: Radiotherapy with or without hyperthermia in the treatment of superficial localized breast cancer. Results from five randomized controlled trials. Int J Radiat Oncol Biol Phys 35:731-744, 1996.

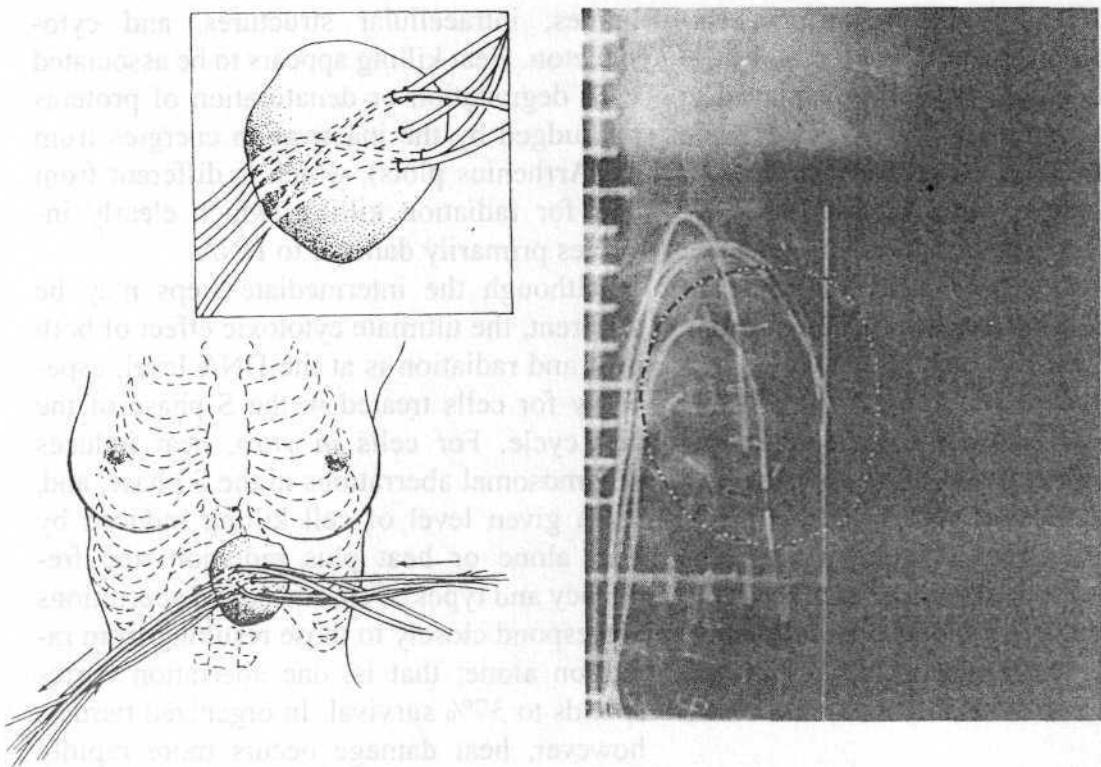
<sup>e</sup>Sneed PK, Stauffer PR, Diederich CJ, et al.: Survival benefit of hyperthermia in a prospective randomized trial of brachytherapy boost ± hyperthermia for glioblastoma multiforme. 38th Am. Soc. Therap. Radiol. Oncol., Los Angeles, CA, October, 1996, (Abs 1).

<sup>f</sup>Van der Zee J, Gonzalez Gonzalez D, Vernon CC, et al.: Therapeutic gain by hyperthermia added to radiotherapy. ICRO/OGRO 6, 6th Int. Meeting on Progress in Radio-Oncology, Salzburg, Austria, May 13-17, 1998.

ing the protracted treatment time. Because heat inhibits the repair of sublethal damage, it is logical to predict that the **effectiveness** of low dose rate irradiation would be enhanced by the simultaneous application of heat. This has been investigated in detail *in vitro* and confirmed *in vivo*, with TERs approaching 2. These reports inspired the use of **hyperthermia** and low dose rate irradiation in implants in patients. Inde-

pendent of the method used to produce the heating, some remarkable results have been obtained with interstitial heating combined with brachytherapy. The reason for this superior response is likely to be the relatively homogeneous heating distribution, in general far better than can be achieved by external heating.

Figure 28.14 shows the technique of combining hyperthermia with a low dose rate im-



**Figure 28.14.** The use of a combination of hyperthermia and low dose rate irradiation for the treatment of retrogastric squamous cell carcinoma. **Left:** Standard 14-gauge brachytherapy catheters surgically implanted during an exploration laparotomy. Once exteriorized, they function as conduits through which radioactive iridium-192 wires or microwave antennae can be inserted. **Right:** Anterior radiograph showing the microwave antennae in place. (From Coughlin CT, Wong TZ, Strohbehn JW, et al.: Intraoperative interstitial microwave-induced hyperthermia and brachytherapy. *Int J Radiat Oncol Biol Phys* 11:1673-1678, 1985, with permission.)

plant of indium-192 wires. Brachytherapy catheters are implanted surgically and function as conduits for radioactive sources or microwave antennae. Results of these combined treatments are impressive, although no controlled study has been performed and it is not clear whether the results are better than could have been obtained with a conventional implant of radium needles or iridium-192 wires. Implants with radioactive sources give good localized dose-distributions. In addition, heating by means of implanted electrodes or antennae gives a better heat distribution than usually can be obtained by external microwave or ultrasonic devices. If the best heat distribution is combined with the best localized radiation dose distribution, it should come as no surprise that the clinical results are good. More recently, good results have been claimed for the combination of hyper-

thermia and high dose rate implants. This tends to confirm the impression that the success is a result of the favorable physical distributions of both heat and radiation that are possible with implants rather than of any particular biologic advantage.

#### POSTSCRIPT ON HYPERTHERMIA

Research in hyperthermia has dropped out of the limelight in recent years, despite the demonstration in several clinical trials summarized previously that it can enhance the effect of radiation therapy in a number of sites. The position can be summed up as follows:

1. The biologic properties of hyperthermia make it an attractive modality for the treatment of cancer, suggesting a useful differential between effects on tumors and normal tissues.

2. It is still difficult to achieve uniform heating of a volume deep within the body, despite considerable ingenuity displayed in designing heating systems. The basic laws of physics make the desired end difficult to achieve.
3. The clinical value of hyperthermia in the routine treatment of cancer is still not clear, despite its proven efficacy in a few specific instances. It should be noted that the situations in which hyperthermia appears to be effective involve either combination with external-beam radiotherapy for the treatment of superficial tumors—in which adequate heating is not so difficult—or combination with brachytherapy, in which heating is induced by implanted electrodes, inevitably involving all the complications and limitations of accessibility.

### MECHANISMS OF ACTION OF HYPERHERMIA

Hyperthermia induces effects in both the nucleus and the cytoplasm. It is unclear whether heat effects in the nucleus, such as loss of polymerase activities, inhibition of DNA synthesis, and chromosomal aberrations, are direct effects of heat within the nucleus or secondary to the effects on the mem-

branes, intracellular structures, and cytoskeleton. Heat killing appears to be associated with degradation or denaturation of proteins (as judged by the inactivation energies from the Arrhenius plots), which is different from that for radiation killing, which clearly involves primarily damage to DNA.

Although the intermediate steps may be different, the ultimate cytotoxic effect of both heat and radiation is at the DNA level, especially for cells treated in the S phase of the cell cycle. For cells *in vitro*, heat induces chromosomal aberrations in the S phase, and, for a given level of cell killing induced by heat alone or heat plus radiation, the frequency and types of chromosomal aberrations correspond closely to those resulting from radiation alone; that is, one aberration corresponds to 37% survival. In organized tissues, however, heat damage occurs more rapidly than radiation damage, because differentiated cells are killed as well as dividing cells. Also, cells tend to die in apoptosis.

The events associated with heat radiosensitization involve DNA damage and the inhibition of its repair. Hyperthermia has little effect on the amount of radiation-induced DNA damage in terms of single- or double-strand breaks. The role of heat appears to be to block the process of repair of these radiation-induced lesions.

### SUMMARY OF PERTINENT CONCLUSIONS

- Localized hyperthermia using external devices can be induced by microwaves, ultrasound, or radiofrequency-induced currents.
- The best heat distributions can be obtained by implanting microwave or radiofrequency sources.
- Heat kills: Survival curves for heat are similar in shape to those for radiation, except that time at the elevated temperature replaces absorbed dose.
- The **inactivation** energy is different above and below a break temperature of 42.5 to 43°C; that is, change of sensitivity with temperature is more rapid below than above this temperature.
- Above the break point, the heating time required to produce a given level of cell killing is halved for every 1-degree temperature rise; below the break point, the time must be reduced by a factor of 4 to 6 for each 1-degree temperature rise.

- Different cells have very different sensitivities to heat. No consistent difference in inherent sensitivity exists between normal and malignant cells.
- The age-response function for heat complements that for x-rays. S-phase cells that are resistant to x-rays are sensitive to heat.
- Cells at low pH and nutritionally deprived (more likely to be in tumors) are more sensitive to heat, although cells can adapt to pH changes in 80 to 100 hours and lose their sensitivity to heat.
- Hypoxia does not protect cells from heat as it does from x-rays.
- Heat damage in normal tissues and tumors is expressed more rapidly than x-ray damage, because heat kills differentiated as well as dividing cells, and also cells can die in interphase rather than at the next (or subsequent) mitosis, as in the case of x-rays.
- Thermotolerance is the induced resistance to a second heat exposure by prior heating.
- Thermotolerance can be induced during a heat exposure at temperatures below about 42.5°C but develops some hours after heating for temperatures above 42.5°C.
- The development of thermotolerance may be monitored by the appearance of heat-shock proteins.
- Thermotolerance is a complication in fractionated clinical hyperthermia. With our present state of knowledge the best way to deal with it may be to avoid it.
- Heat preferentially damages tumor vasculature. After heating, blood flow goes down in tumors but increases in normal tissues. This may result in an enhanced temperature differential between tumors and normal tissues. There is good evidence for this in transplantable animal tumors, but it is less clear in spontaneous human tumors.
- Mild hyperthermia can promote tumor reoxygenation; this has been shown in animal experiments and in clinical studies.
- Temperature measurement *in vivo* is difficult but improving with multiple nonmetallic thermometers. The most successful noninvasive technique involves magnetic resonance imaging.
- Thermal dose is expressed in terms of cumulative equivalent minutes at 43°C.
- In experimental animals treated with heat alone, tumor cure correlates best with *minimum temperature*. If heat and radiation are combined, however, tumor response correlates best with some measure of temperature distribution.
- Delivered simultaneously, hyperthermia interacts with radiation in a more-than-additive way and inhibits the repair of sublethal damage.
- The thermal enhancement ratio (TER) is the ratio of radiation doses with and without heat to produce the same biologic effects. TERs of 2 to 4 can be obtained in tumors and normal tissues in experimental animals.
- The therapeutic gain factor is the ratio of the TER in the tumor to the TER in normal tissues.
- In a clinical situation, in which modest levels of hyperthermia are attained and small daily doses of radiation are delivered, the cytotoxic effects of the two modalities are probably independent and additive.
- Hyperthermia potentiates some chemotherapeutic agents. Local hyperthermia "targets" their action.
- Many thousands of patients have been treated with hyperthermia.
- The general consensus is that hyperthermia alone is of limited use, except for palliation or re-treatment of recurrent tumors.
- Several clinical studies have shown that the combination of hyperthermia and radiation produces more complete and partial tumor responses than radiation alone.

- Several phase III randomized trials show a clear and significant benefit for the addition of hyperthermia to standard radiation therapy. The sites include superficial localized breast cancer, recurrent or metastatic melanoma, and nodal metastases from head-and-neck cancer.
- Trials involving advanced breast cancer and neck nodes with full-dose radiation therapy did not show any benefit from the addition of hyperthermia.
- Biologic properties of heat appear favorable for its use in cancer therapy. Physical devices to produce uniform heating at a depth are not well developed, and adequate thermometry is also difficult.
- Microwaves provide good localization at shallow depths, but localization is poor at greater depths, at which low frequencies are needed.
- With ultrasound, deep heating can be achieved with focused arrays, but bones or air cavities cause distortions.
- In practice, breast tumors, including recurrences and melanomas, can be heated adequately with microwaves, and deep-seated tumors below the diaphragm can be heated adequately with ultrasound. In any other site present methods of heating pose a problem.
- The combination of interstitial implants of radioactive sources with hyperthermia generated by radiofrequency power applied to the implanted sources appears to be particularly promising, because a good radiation dose distribution is combined with a good heat distribution.
- Hyperthermia induces effects in both the nucleus and the cytoplasm. Probable mechanisms for heat killing include damage to the plasma membrane and inactivation of proteins, but these mechanisms may be different above and below the break temperature in the Arrhenius plot.
- Heat radiosensitization involves DNA damage and its repair.

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## Glossary

**A** Symbol for mass number.

**absolute risk** The risk of an adverse health effect that is independent of other causes of that same health effect.

**absorbed dose (D)** The energy imparted per unit mass by ionizing radiation to matter at a specific point. The SI unit of absorbed dose is joule per kilogram (J/kg). The special name for this unit is gray (Gy). The previously used special unit of absorbed dose, the rad, was defined to be an energy absorption of 100 erg/g. Thus, 1 Gy = 100 rad.

**absorption** Removal of x-rays from a beam.

**accelerator** A device for increasing the velocity and the energy of charged elementary particles, for example, electrons or protons, through application of electric forces or magnetic forces or both of them. Accelerators have made particles move at velocities approaching the speed of light.

**accelerator (linear)** A machine, often called a *linac*, that produces high-energy x-rays for the treatment of cancer.

**accelerated fractionation** Reduction in overall treatment time; a schedule in which the average rate of dose delivery exceeds the equivalent of 10 Gy (1,000 rad) per week in 2-Gy (200-rad) fractions.

**acidic fibroblast growth factor (FGF)** A mitogen for many types of cells of mesodermal origin, including endothelial cells, chondrocytes, and fibroblasts. In presence of heparin, induces blood vessel growth.

**action level** Concentration of radon in a house above which it is recommended that

some action be taken to reduce that radon level; currently, the action **level** is 4 pCi/l in the United States.

**activation** The process of making a material radioactive by bombardment with neutrons, protons, or other nuclear particles. See **activation analysis, induced radioactivity**.

**activation analysis** Identifying and measuring chemical elements in a sample of material. First, the sample is made radioactive by bombardment with neutrons, charged particles, or gamma rays. Then, the newly formed radioactive atoms **in** the sample give off characteristic nuclear radiations, such as gamma rays, which tell the types and quantities of atoms present. Activation analysis is usually more sensitive than chemical analysis.

**activity** Quantity of a radionuclide that describes the rate at which decays occur in an amount of a radionuclide. The SI unit of radioactivity is the becquerel (Bq), which replaced the old unit, the curie (Ci). One becquerel corresponds to one disintegration of a radionuclide per second.

**additive** A situation in which the effect of a combination is the sum of the effects of the separate treatments (**independent** cell kill).

**adjuvant therapy** A treatment method used in addition to the primary therapy. Radiation therapy often is used as an adjuvant to surgery or chemotherapy.

**agarose gel electrophoresis** A matrix composed of purified agar is used to separate

larger DNA and RNA molecules ranging from 100 to 20,000 nucleotides.

**ALARA (as low as reasonably achievable)**

The principle of limiting the radiation dose of exposed persons to levels as low as are reasonably achievable, economic and social factors being taken into account.

**allele** One of the possible mutational states of a gene, distinguished from other alleles by phenotypic effects.

**alleles** Alternate forms of a gene or DNA sequence on the two homologous chromosomes of a pair.

**alopecia** Hair loss.

**alpha/beta ratio** The ratio of the parameters  $\alpha$  and  $\beta$  in the linear-quadratic model; used to quantify the fractionation sensitivity of tissues.

**alpha fetoprotein (AFP)** A 70-kd glycoprotein synthesized in embryonic development by the yolk sac. High levels of this protein in the amniotic fluid are associated with neural tube defects such as spina bifida. Lower than normal levels may be associated with Down's syndrome.

**alpha particle** A positively charged particle emitted by radioactive materials. It consists of two neutrons and two protons bound together; hence, it is identical with the nucleus of a helium atom. It is the least penetrating of the three common types of radiation—alpha, beta, and gamma—and it is stopped by a sheet of paper. It is not dangerous to plants, animals, or humans unless the  $\alpha$ -emitting substance has entered the organisms.  $\alpha$ -Particles are ejected from a nucleus during the decay of some radioactive elements: for example, an  $\alpha$ -particle is emitted if either of the radon progeny polonium-218 or polonium-214 decays.

**alpha ray** A stream of  $\alpha$ -particles. Used loosely as a synonym for  $\alpha$ -particles.

**Alu** sequence An interspersed DNA sequence of approximately 300 bp found in the genome of primates that is cleaved by the restriction enzyme *Alu* I. *Alu* sequences are composed of a head-to-tail dimer, with the first monomer approximately 140 bp and the second approximately 170 bp. In humans, they are dispersed throughout the

genome and are present in 300,000 to 600,000 copies, constituting some 3 to 6% of the genome. See SINES.

**Ames test** A procedure devised by Bruce Ames to test carcinogenic properties of chemicals by their ability to induce mutations in the bacterium *Salmonella*.

**amino acid** Any of the 20 subunit building blocks that are covalently linked to form proteins.

**ampicillin** An antibiotic that prevents bacterial growth.

**amplify** To increase the number of copies of a DNA sequence by inserting into a cloning vector that replicates within a host cell *in vivo* or *in vitro* by the polymerase chain reaction.

**analogue** A chemical compound structurally similar to another, but differing by a single functional group (e.g., 5-bromo-deoxyuridine is an analogue of thymidine).

**anaphase** Stage of cell division in which chromosomes begin moving to opposite poles of the cell.

**anemia** Having too few red blood cells. Symptoms of anemia include feeling tired, weak, and short of breath.

**aneuploidy** A condition in which the chromosome number is not an exact multiple of the haploid set.

**angiography** The radiographic visualization of blood vessels following introduction of contrast material.

**angioplasty** Reconstruction of blood vessels.

**angstrom** (A) A unit of length equal to  $10^{-10}$  meters.

**anneal** The pairing of complementary DNA or RNA sequences *via* hydrogen bonding to form a double-stranded polynucleotide.

**anode** Positive side of the x-ray tube; contains the target.

**anorexia** Poor appetite.

**antibiotic** A compound that inhibits the growth of or kills microorganisms.

**antibiotic resistance** The ability of a microorganism to disable an antibiotic or prevent transport of the antibiotic into the cell.

**antibody** Protein (immunoglobulin) produced in response to an antigenic stimulus

with the capacity to bind specifically to the antigen.

**antiemetic** A drug used to control nausea and vomiting.

**antigen** Any foreign substance that elicits an immune response by stimulating the production of antibodies.

**antiparallel** Describing molecules in parallel alignment, but running in opposite directions. Most commonly used to describe the opposite orientations of the two strands of a DNA molecule.

**apoptosis** A mode of rapid cell death after irradiation in which the cell nucleus displays characteristic densely staining globules and at least some of the DNA is subsequently broken down into internucleosomal units. Sometimes postulated to be a "programmed," and therefore potentially controllable, process.

**asexual reproduction** Production of offspring in the absence of any sexual process.

**atom** A particle of matter indivisible by chemical means. It is the fundamental building block of the chemical elements, which differ from each other because they contain different kinds of atoms. According to present theory, an atom contains a dense inner core, the nucleus, and a much less dense outer domain consisting of electrons in motion around the nucleus. Atoms are electrically neutral. Compare **ion**, **molecule**. See **matter**.

**atomic energy** See **nuclear energy**.

**atomic mass** See **atomic weight, mass**.

**atomic mass unit (amu)** One sixteenth the mass of a neutral atom of the most abundant isotope of oxygen,  $^{16}\text{O}$ . See **atomic weight, mass number**.

**atomic number (Z)** The number of protons in the nucleus of an atom and also its positive charge.

**atomic weight (at. wt.)** The mass of an atom relative to other atoms. The present-day basis for the scale of atomic weights is oxygen; the commonest isotope of this element arbitrarily has been assigned an atomic weight of 16. The unit of the scale is 1/16 the weight of the  $^{16}\text{O}$  atom, or roughly the mass of one proton or one neutron. The

atomic weight of any element is approximately equal to the total number of protons and neutrons in its nucleus. Compare **atomic number**.

**ATP** Adenosine triphosphate.

**autoimmune disease** The production of antibodies that results from an immune response to one's own molecules, cells, or tissues. Such a response results from the inability of the immune system to distinguish self from nonself. Diseases such as arthritis, scleroderma, systemic lupus erythematosus, and perhaps diabetes are considered to be autoimmune diseases.

**autoradiography** Use of a photographic emulsion to detect the distribution of a radioactive label in a tissue specimen.

**autosomes** Chromosomes other than the sex chromosomes. In humans, there are 22 pairs of autosomes.

**B lymphocyte** A white blood cell responsible for production of antibodies involved **in** the humoral immune response.

**background radiation** The radiation in the natural environment, including cosmic rays and radiation from the naturally radioactive elements, both outside and inside the bodies of humans and animals. It also is called *natural radiation*. The term also may mean radiation that is unrelated to a specific experiment.

**bacteriophage** A virus that infects bacteria. Altered forms are used as vectors for cloning DNA.

**bacterium** A single-cell prokaryotic organism.

**basal cells** Cells at the base of the wall of the lung airways. These cells divide to replenish the other cells in the lung wall and often are considered the key cells that, if damaged, can lead to lung cancer.

**base pair** A pair of complementary nitrogenous bases in a DNA molecule: adenine-thymine and guanine-cytosine. It is also a measure of the length of DNA.

**basic fibroblast growth factor (FGF)** A mitogen for many types of cells of mesodermal or neuroectodermal origin. Has a variety of angiogenic properties.

**B-DNA** See **double helix**.

**beam** A stream of particles or electromagnetic radiation moving in a single direction.

**becquerel (Bq)** Unit of radioactivity, corresponding to one radioactive disintegration per second. See **activity**.

**benign** Describing a slow-growing, not malignant, tumor that does not spread to other parts of the body. If completely removed, benign lesions do not tend to recur. Incompletely removed tumors may recur but do not spread. Although benign, these tumors may cause permanent damage to some structures in the brain.

**beta particle (P)** An elementary particle emitted from a nucleus during radioactive decay, with a single electric charge and a mass equal to 1/1837 of that of a proton. A negatively charged ( $\beta$ -particle) is identical to an electron. A positively charged ( $\beta$ -particle) is called *a positron*.  $\beta$ -Radiation may cause skin burns, and ( $\beta$ -emitters are harmful if they enter the body.  $\beta$ -Particles, however, are stopped easily by a thin sheet of metal.

**BeV** One billion electron volts. Also written *GeV*.

**bilateral** On both sides of the body.

**biochemistry** Chemical reactions that sustain life.

**biologic half-life** See **half-life, biologic**.

**biologic therapy** Treatment to stimulate or restore the ability of the immune system to fight infection and disease. Also called *immunotherapy*.

**biologically effective dose (BED)** In fractionated radiotherapy, the total dose that would be required in very small dose fractions to produce a particular effect, as indicated by the linear-quadratic equation. Otherwise known as "extrapolated total dose." BED values calculated for different *oc/p* ratios are not strictly comparable.

**biopsy** The removal of a small portion of a tumor to allow a pathologist to examine it under a microscope and provide a diagnosis of tumor type.

**biotechnology** Commercial or industrial processes that utilize biologic organisms or products.

**blood count** The number of red blood cells, white blood cells, and platelets in a sample of blood.

**body burden** The amount of radioactive material present in a human or an animal. See **background radiation, whole-body counter**.

**bone marrow** Spongy tissue in the cavities of large bones, in which the body's blood cells are produced.

**bone seeker** A radioisotope that tends to accumulate in the bones, for example, the strontium-90 isotope, which behaves chemically like calcium.

**brachytherapy** Internal radiation treatment achieved by implanting radioactive material directly into the tumor or very close to it. Sometimes called *internal radiation therapy*.

**breeder reactor** A nuclear reactor that produces fissionable fuel as well as consuming it, especially one that creates more than it consumes. The new fissionable material is created by neutron capture in fertile materials. The process by which this occurs is known as *breeding*.

**Bremsstrahlung x-rays** X-rays resulting from interaction of the projectile electron with a target nucleus; braking radiation.

**5-bromodeoxyuridine (BrdU)** A mutagenically active analogue of thymidine in which the methyl group at the 6' position in thymine is replaced by bromine.

**byproduct material** Any radioactive material (except source material or fissionable material) obtained during the production or the use of source material or fissionable material. Byproduct material includes fission products and many other radioisotopes produced in nuclear reactors.

**cyclic adenosine monophosphate (cAMP)** An important regulatory molecule in both prokaryotic and eukaryotic organisms.

**cancer** A general name for more than one hundred diseases in which abnormal cells grow out of control; a malignant tumor.

**carcinogen** A physical or chemical agent capable of causing cancer, such as radon progeny, cigarette smoke, or asbestos.

**carcinogenic** A property of some agent (for example, smoke or alcohol) that could contribute to the development of cancer (same as **oncogenic**).

**CAT scan** Computerized axial tomography, often called a *CT scan*, which provides three-dimensional x-ray images of some part of the body. It is useful for diagnosing cancer and for planning radiation therapy treatments.

**cathode** Negative side of the x-ray tube; contains the filament and focusing cup.

**cdc2 (also known as cdk1)** Kinase that associates with cyclin B to regulate entry into mitosis. Complex is activated by Cdc25-mediated dephosphorylation. Also associates with cyclin A during M phase.

**cdc25C** Phosphatase that activates the cyclin B-Cdc2 complex.

**cdk2** Kinase which associates with cyclin E during the G<sub>1</sub>/S transition, and cyclin A during S phase. Inhibited by p21 and p27.

**cdk4** Kinase that associates with cyclin D1. Complex can phosphorylate pRb to allow cells to progress through G<sub>1</sub>. Inhibited by p16, p21, and p27.

**cdks (cyclin dependent kinases)** Proteins that complex with their cyclin regulatory subunits to phosphorylate proteins necessary for progression through the cell cycle.

**cDNA (copy DNA)** DNA synthesized from an RNA template using reverse transcriptase.

**cDNA library** A library composed of complementary copies of cellular mRNAs (*i.e.*, the exons without the introns).

**cell** The basic unit of living matter.

**cell cycle** Sum of the phases of growth of an individual cell type; divided into G<sub>1</sub> (gap 1), S (DNA synthesis), G<sub>2</sub> (gap 2), and M (mitosis); the cycle of cellular events from one mitosis to the next.

**cell-cycle time** The time between one mitosis and the next.

**cell-loss factor (0)** The rate of cell loss from a tumor, as a proportion of the rate at which cells are being added to the tumor by mitosis. Usually calculated by the relation  $0 = 1 - T_{po}/T_d$ , in which  $T_{po}$  is potential

doubling time and T<sub>d</sub> is the cell population doubling time.

**cells** The body is made up of tiny, functioning units called *cells*. These can be observed under a microscope. Each cell plays a specialized role in the body. Groups of cells are organized together to form tissue. Tissues are organized to form organs in the body.

**cellular oncogene (protooncogene)** A normal gene that if mutated or improperly expressed contributes to the development of cancer.

**centimorgan** A unit of distance between genes on chromosomes. One centimorgan represents a value of 1% crossing over between two genes.

**central nervous system (CNS)** Refers to the brain and spinal cord.

**centriole** A cytoplasmic organelle composed of nine groups of microtubules, generally arranged in triplets. Centrioles function in the generation of cilia and flagella and serve as foci for the spindles in cell division.

**centromere** The chromosome constriction to which the spindle fiber attaches. The position of the centromere determines whether chromosomes are metacentric (X-shaped; *e.g.*, chromosomes 1, 3, 16, 19, 20) or acrocentric (inverted V-shaped; *e.g.*, chromosomes 13-15, 21, 22, Y). During mitosis the identical chromatids of each chromosome are separated by shortening of the spindle fibers attached to opposite poles of the dividing cell.

**c-fv/-B2/HER2/c-neu** A gene closely related to the epidermal growth factor receptor, which is amplified in a variety of cancers including that of the breast.

**cervix** The lower part of the uterus, which projects out into the vagina.

**c-Jbs** An early response gene induced by mitogenic stimuli. Forms complexes in the nucleus which act as transcription factors. Recognizes AP-1 sites if complexed with c-jun.

**chain reaction** A reaction that stimulates its own repetition. In a fission chain reac-

tion, a fissionable nucleus absorbs a neutron and fissions, releasing additional neutrons. These, in turn, can be absorbed by other fissionable nuclei, releasing still more neutrons. A fission chain reaction is self-sustaining if the number of neutrons released in a given time equals or exceeds the number of neutrons lost by absorption in nonfissioning material or by escape from the system.

**characteristic x-rays** X-rays produced following ionization of inner-shell electrons; characteristic of the target element.

**charged particle** An ion; an elementary particle that carries a positive or negative electric charge.

**chemotherapist** A physician who specializes in the use of drugs to treat cancer, now called a *medical oncologist*.

**chemotherapy** Treatment with anticancer drugs.

**chi-square ( $\chi^2$ ) analysis** Statistical test to determine if an observed set of data fits a theoretic expectation.

**chromatid** One of the longitudinal subunits of a replicated chromosome, joined to its sister chromatid at the centromere.

**chromatin** Term used to describe the complex of DNA, RNA, histones, and nonhistone proteins that make up chromosomes.

**chromatography** Technique for the separation of a mixture of solubilized molecules by their differential migration over a substrate.

**chromosomal aberration** Any change resulting in the duplication, deletion, or rearrangement of chromosomal material.

**chromosomal instability** An effect of irradiation in which chromosomal aberrations continue to appear through many cell generations.

**chromosomal mutation** See **chromosomal aberration**.

**chromosomal polymorphism** Alternate structures or arrangements of a chromosome that are carried by members of a population.

**chromosome** In prokaryotes, an intact DNA molecule containing the genome; in eukaryotes, a DNA molecule complexed

with RNA and proteins to form a thread-like structure containing genetic information arranged in a linear sequence.

**chromosome banding** Technique for the differential staining of mitotic or meiotic chromosomes to produce a characteristic banding pattern or selective staining of certain chromosomal regions such as centromeres, the nucleolus organizer regions, and GC- or AT-rich regions. Not to be confused with the banding pattern present in unstained polytene chromosomes, which is produced by the alignment of chromosomes.

**chromosome map** A diagram showing the location of genes on chromosomes.

**chronic** Persisting for a long time.

**chronic hypoxia** Persistent low oxygen concentrations such as exists in viable tumor cells close to regions of necrosis.

**c-jun** An early-response gene induced by mitogenic stimuli and stress responses. Forms complexes in the nucleus that act as transcription factors. Recognizes AP-1 sites if complexed with *c-fos*.

**classical scattering** Scattering of x-rays with no loss of energy.

**clinical trials** Medical research studies conducted with volunteers. Each study is designed to answer scientific questions and to find better ways to prevent or treat cancer.

**clone** Genetically identical cells or organisms all derived from a single ancestor by asexual or parasexual methods. For example, a DNA segment that has been enzymatically inserted into a plasmid or chromosome of a phage or a bacterium and replicated to form many copies.

**cloned library** A collection of cloned DNA molecules representing all or part of an individual's genome.

**clonogenic cells** Cells that have the capacity to produce an expanding family of descendants-(usually at least 50). Also called "colony-forming cells" or "clonogens."

**c-myc** An early response gene induced by mitogenic stimuli, as well as TGF(3). Is highly overexpressed as a result of translocations in Burkitt's lymphomas. Gene is

amplified in certain cancers, as is its relative, N-myc.

**cobalt-60** A radioactive substance used as a radiation-source to treat cancer.

**code** See **genetic code**.

**codon** A group of three nucleotides that specifies the addition of one of the 20 amino acids during translation of mRNA into a polypeptide.

**colchicine** An alkaloid compound that inhibits spindle formation during cell division. Used **in** the preparation of karyotypes to collect a large population of cells inhibited at the metaphase stage of mitosis.

**collective dose** Usually refers to "collective effective dose" obtained by multiplying the average effective dose by the number of persons exposed to that given dose. Expressed **in** person-sieverts. The old unit was the man-rem.

**colon** Large intestine.

**colony** A group of identical cells derived from a single ancestor cell.

**combination chemotherapy** The use of more than one drug to treat cancer.

**complementarity** Chemical affinity between nitrogenous bases as a result of hydrogen bonding. Responsible for the base pairing between the strands of the DNA double helix.

**complementary nucleotides** Members of the pairs adenine-thymine, adenine-uracil, and guanine-cytosine that have the ability to hydrogen bond to one another.

**complementation** Identification of whether a (radiosensitive) phenotype in different mutants is due to the same gene. Studied by means of cell fusion.

**complementation test** A genetic test to determine whether two mutations occur within the same gene. If two mutations are introduced into a cell simultaneously and produce a wild-type phenotype (*i.e.*, they complement each other), they are often nonallelic. If a mutant phenotype is produced, the mutations are noncomplementing and are often allelic.

**Compton effect** Scattering of x-rays resulting in ionization and loss of energy; the en-

ergy lost by the photon is given to the ejected electron as kinetic energy.

**concordance** Pairs or groups of individuals identical in their phenotype. In twin studies, a condition in which both twins exhibit or fail to exhibit a trait under investigation.

**conduction** Transfer of heat by molecular agitation.

**conductor** Material that allows heat or electric current to flow.

**connective tissue** The tissues of the body that bind together and support various structures of the body. Examples are bone, cartilage, and muscle.

**contrast agent** A chemical that is used to highlight disease processes on x-ray tests, contrasting them against the background of normal tissues.

**control group** A group of people subject to the same conditions as another group under study, except that the control group is not exposed to the specific factor being investigated in the study group.

**cosmic rays** Radiation of many sorts, but mostly protons and heavier atomic nuclei with very high energies originating outside the earth's atmosphere. Cosmic radiation is part of the natural background radiation. Some cosmic rays are more energetic than any manmade forms of radiation.

**cosmid** A vector designed to allow cloning of large segments of foreign DNA (25,000<sup>–</sup>5,000 base pairs). Cosmids are hybrids composed of the cos sites of lambda inserted into a plasmid. In cloning, the recombinant DNA molecules are packaged into phage protein coats, and after infection of bacterial cells, the recombinant molecule replicates and can be maintained as a plasmid.

**covalent bond** A nonionic chemical bond formed by the sharing of electrons.

**critical** Capable of sustaining a chain reaction. See **critically**.

**critical assembly** An assembly of sufficient fissionable material and moderator to sustain a fission chain reaction at a very low-power level, permitting study of the assembly's components for various fissionable

materials in different geometrical arrangements.

**critical mass** The smallest mass of fissionable material that supports a self-sustaining chain reaction under stated conditions.

**criticality** The state of a nuclear reactor if it is sustaining a chain reaction.

**cross-section** (a) A measure of the probability that a nuclear reaction will occur. Usually measured in barns, it is the apparent or effective area presented by a target nucleus or particle to an oncoming particle or other nuclear radiation, such as a photon of  $\gamma$ -radiation.

**crossing over** The exchange of chromosomal material (parts of chromosomal arms) between homologous chromosomes by breakage and reunion. The exchange of material between nonsister chromatid during meiosis is the basis of genetic recombination.

**CT scan (CAT scan or CT x-ray)** A three-dimensional x-ray. CT stands for *computerized tomography*.

**cure** An outcome of treatment that leaves the patient disease free, with no likelihood of recurrence.

**curie (Ci)** Old unit of radioactivity, corresponding to  $3.7 \times 10^{10}$  radioactive disintegrations per second. Now replaced by the becquerel.

**cyclin A** Regulatory protein that associates with cdk2 during the S phase of the cell cycle.

**cyclin B** Regulatory protein that associates with cdc2 to regulate entry into mitosis.

**cyclin E** Associates with cdk2 during late G<sub>1</sub> phase, thought to regulate entry into S phase.

**cyclin D1** One of three **cyclin D** family members (also D2, D3). Associates with Cdk5 or Cdk6 to regulate through the Q<sub>1</sub> phase of the cell cycle. Is induced by a variety of mitogens. Gene is amplified in breast, esophageal, and other types of tumors.

**cyclins** Proteins that complex with cdks to regulate progression through the cell cycle.

**cyst** A cavity, usually filled with a liquid, sometimes associated with benign or malignant tumors.

**cytogenetics** A branch of biology in which the techniques of both cytology and genetics are used to study heredity.

**cytokines** Polypeptides, originally defined as being released from lymphocytes and involved in maintenance of the immune system. These factors have pleiotropic effects on not only hematopoietic cells but many other cell types as well.

**Do** A parameter in the multitarget equation: the radiation dose that reduces survival to  $e^{-1}$  (*i.e.*, 0.37) of its previous value on the exponential portion of the survival curve.

**dalton** A unit of mass equal to that of the hydrogen atom, which is  $1.67 \times 10^{-24}$  gram. A unit used in designating molecular weights.

**daughter** A nuclide formed by the radioactive decay of another nuclide, which in this context is called the *parent*. Replaced by *progeny*.

**decay chain** A radioactive series.

**decay series/chain** A series of radioactive atoms, each the "progeny" of the one before and the "parent" of the one after; the series ends if any daughter is not radioactive.

**decay, radioactive** The spontaneous transformation of one nuclide into a different nuclide or into a different energy state of the same nuclide. The process results in a decrease, with time, of the original radioactive atoms in a sample. Radioactive decay involves the emission from the nucleus of  $\alpha$ -particles,  $\beta$ -particles (electrons), or  $\gamma$ -rays; the nuclear capture or ejection of orbital electrons; or fission. Also called *radioactive disintegration*. See **half-life**, **nuclear reaction**, **radioactive series**.

**deletion** A segment of a chromosome is missing as a result of two breaks and loss of the intervening piece.

**deoxyribonucleic acid (DNA)** A macromolecule usually consisting of antiparallel polynucleotide chains held together by hy-

drogen bonds, in which the sugar residues are deoxyribose. The primary carrier of genetic information.

**dermatitis** A skin rash.

**deterministic effect** An effect for which the severity of the effect in affected individuals varies with the dose, and for which a threshold usually exists.

**deuterium ( $^2\text{H}$  or D)** An isotope of hydrogen whose nucleus contains one neutron and one proton and, therefore, is about twice as heavy as the nucleus of normal hydrogen, which is only a single proton. Deuterium often is referred to as *heavy hydrogen*: it occurs in nature as 1 atom to every 6,500 atoms of normal hydrogen. It is non-radioactive. Compare **tritium**. See **heavy water**.

**deuteron** The nucleus of deuterium. It contains one proton and one neutron.

**dicentric chromosome** A chromosome having two centromeres.

**differential diagnosis** A list of the most likely diagnoses for a particular set of symptoms and x-ray findings. The use of different imaging techniques often narrows the differential diagnosis to the most likely disease present.

**differentiation** The process of complex changes by which cells and tissues attain their adult structure and functional capacity.

**digest** To cut DNA molecules with one or more restriction endonucleases.

**diploid** A condition in which each chromosome exists in pairs; having two of each chromosome.

**direct action** Ionization or excitation of atoms within DNA leading to free radicals, as distinct from the reaction with DNA of free radicals formed in nearby water molecules.

**disjunction** The separation of chromosomes at the anaphase stage of cell division.

**diuretics** Drugs that help the body get rid of excess water and salt.

**dizygotic twins** Twins produced from separate fertilization events; two ova fertilized

independently. Also known as *fraternal twins*.

**DNA (deoxyribonucleic acid)** An organic acid composed of four nitrogenous bases (adenine, thymine, cytosine, and guanine) linked via sugar and phosphate units. DNA is the genetic material of most organisms and usually exists as a double-stranded molecule in which two antiparallel strands are held together by hydrogen bonds between adenine-thymine and cytosine-guanine base pairs.

**DNA fingerprint** A unique pattern of DNA fragments identified by Southern hybridization or by polymerase chain reaction.

**DNA polymerase** An enzyme that catalyzes the synthesis of DNA from deoxyribonucleotides and a template DNA molecule.

**DNA polymorphism** One or two or more alternate forms of a chromosomal locus that differ in nucleotide sequence or have variable numbers of repeated nucleotide units.

**DNA sequencing** Procedures for determining the nucleotide sequence of a DNA fragment.

**DNase (deoxyribonucleosidase)** An enzyme that degrades or breaks down DNA into fragments or constitutive nucleotides.

**dominance** The expression of a trait in the heterozygous condition.

**dominant gene** A gene whose phenotype is expressed if it is present in a single copy.

**dose** General term for the quantity of radiation. See **absorbed dose**, **equivalent dose**, **effective dose**, **collective effective dose**.

**dose rate** The radiation dose delivered per unit time and measured, for instance, in grays per hour.

**dose-rate effect** Decreasing radiation response with decreasing radiation dose rate.

**dose-modifying factor (DMF)** If a chemical or other agent acts as if to change the dose of radiation, DMF indicates the ratio of dose without to dose with the agent for the same level of effect. Similarly: **dose-re-**

**duction factor or sensitizer enhancement ratio.**

**dosimetrist** A person who plans and calculates the proper radiation dose for treatment.

**double trouble** A hot spot within a treatment field receives not only a higher dose but also a higher dose per fraction, which means that the biologic effectiveness of the dose is also greater.

**double helix** The model for DNA structure proposed by James Watson and Francis Crick, involving two antiparallel, hydrogen-bonded polynucleotide chains wound into a righthanded helical configuration, with 10 base pairs per full turn of the double helix. Often called *B-DNA*.

**doubling time** Time for a cell population or tumor volume to double its size.

**early responses** Radiation-induced normal-tissue damage that is expressed in weeks to a few months after exposure. Generally due to damage to parenchymal cells,  $\alpha/\beta$  ratio tends to be large.

**early response gene** A gene such as *c-fos*, *c-jun*, or *c-myc* whose mRNA levels are induced dramatically following mitogenic stimuli or stress.

**ED50 (effect dose 50%)** Radiation dose that produces a specified effect in the normal tissues of 50% of animals.

**edema** Abnormal accumulation of fluid; e.g., pulmonary edema refers to a buildup of fluid in the lungs.

**effective dose** The radiation dose allowing for the fact that some types of radiation are more damaging than others, and some parts of the body are more sensitive to radiation than others. It is defined as the sum over specified tissues of the products of the equivalent dose in a tissue and the weighting factor for that tissue.

**effective dose equivalent** Quantity obtained by multiplying the dose equivalents to a tissue by the appropriate risk weighting factor for that tissue. Expressed in sieverts.

**electromagnetic radiation** Radiation consisting of associated and interacting electric

and magnetic waves that travel at the speed of light, such as light, radio waves,  $\gamma$ -rays, and x-rays. All electromagnetic radiation can be transmitted through a vacuum.

**electron (e)** An elementary particle with a unit negative electrical charge and a mass  $1/1837$  that of the proton. Electrons surround the positively charged nucleus and determine the chemical properties of the atom. Positive electrons, or *positrons*, also exist.

**electron volt (eV)** The amount of energy gained by a particle of charge  $e(-1.6 \times 10^{-19} C)$  if it is accelerated by a potential difference of 1 V. 1 eV =  $1.6 \times 10^{-19}$  J.

**electrophoresis** The technique of separating charged molecules in a matrix to which an electrical field is applied.

**electroporation** High-voltage pulses of electricity are used to open pores in the cell membrane through which foreign DNA can pass.

**endonuclease** An enzyme that hydrolyzes internal phosphodiester bonds in a polynucleotide chain or nucleic acid molecule.

**epidermal growth factor (EGF)** Promotes growth of epidermal cells, can stimulate or inhibit the proliferation and differentiation of a wide variety of cells.

**epithelium** A thin layer of cells in the skin, mucous membrane, or any duct that replaces worn-out cells by cell division.

**equivalent dose** A quantity used for radiation-protection purposes that takes into account the different probability of effects which occur with the same absorbed dose delivered by radiations with different radiation weighting factor values. It is defined as the product of the average absorbed dose in a specified organ or tissue and the radiation weighting factor values. If dose is in grays, equivalent dose is in sieverts.

**erythropoietin** Cytokine that relates erythrocyte levels and stimulates late erythroid progenitors to form small colonies of erythrocytes.

***Escherichia coli*** A bacterium found in the human colon that is used widely as a host for molecular cloning experiments.

**ethidium bromide** A fluorescent dye used to stain DNA and RNA, which intercalates between nucleotides and fluoresces if exposed to ultraviolet light.

**eukaryotes** Those organisms having true nuclei and membranous organelles and whose cells demonstrate mitosis and meiosis.

**evolution** The origin of plants and animals from preexisting types. Descent with modifications.

**excision repair** Repair of DNA lesions by removal of a polynucleotide segment and its replacement with a newly synthesized, corrected segment.

**exon** DNA segment of a gene that is transcribed and translated into protein.

**exonuclease** An enzyme that breaks down nucleic acid molecules by breaking the phosphodiester bonds at the 3' or 5' terminal nucleotides.

**exponential growth** Growth according to an exponential equation:  $V \sim V_0 \exp(kt)$ . The volume doubling time is constant ( $= [\log_2]/k$ ).

**exponential survival curve** A survival curve without a threshold or shoulder region that is a straight line on a semilogarithmic plot.

**exposure (X)** Exposure is used more often in its more general sense and not as the specially defined radiation quantity. A measure of the quantity of x- or y-radiation based upon its ability to ionize air through which it passes. The SI unit of exposure is coulomb per kilogram (C/kg). The previously used special unit of exposure, roentgen (R), has been replaced.  $1 R = 2.58 \times 10^{-4} C/kg$  (exactly). The physical quantity exposure may be replaced by the quantity air kerma in air especially for calibration of monitoring instruments.  $1 R \sim 10 \text{ mGy}$  air kerma.

**expression library** A library of cDNAs whose encoded proteins are expressed by specialized vectors.

**expression vector** Plasmids or phage-carrying promoter regions designed to cause expression of cloned DNA sequences.

**external radiotherapy** Radiation therapy that uses a machine located outside of the body to aim high-energy rays at cancer cells. Sometimes called *external-beam radiotherapy*.

**extrapolated total dose (ETD)** Calculated isoeffect dose when the dose rate is very low or when fraction size is very small. See **biologically effective dose**.

**extrapolation number** A parameter in the multitarget equation: the point on the survival scale to which the straight part of the curve back-extrapolates.

**familial trait** A trait transmitted through and expressed by members of a family.

**fast neutrons** Neutrons with energy greater than approximately 100,000 eV (electron volts). Compare **intermediate neutrons, prompt neutrons, thermal neutrons**.

**field** A term used in radiation oncology to describe or define an area through which x-rays are directed toward the tumor.

**field-size effect** The dependence of normal-tissue damage on the size of the irradiated area; also known as *volume effect*.

**film badge** An assembly containing a packet of unexposed photographic film and a variety of filters (absorbers); when the film is developed, the dose and type of radiation to which the wearer was exposed can be estimated.

**fingerprint** The pattern of ridges and whorls on the tip of a finger. The pattern obtained by enzymatically cleaving a protein or nucleic acid and subjecting the digest to two-dimensional chromatography or electrophoresis.

**FISH (fluorescence *in situ* hybridization)** Fluorescent dyes are attached to specific region of the genome, thus aiding the identification of chromosomal damage.

**fission** The splitting of a heavy nucleus into two approximately equal parts (which are nuclei of lighter elements), accompanied by the release of a relatively large amount of energy and generally one or more neutrons. Fission can occur spontaneously, but usually it is caused by nuclear absorption of y-rays, neutrons, or other particles.

**fission products** The nuclei (fission fragments) formed by the fission of heavy elements plus the nuclides formed by the fission fragments' radioactive decay.

**flanking region** The DNA sequences extending on either side of a specific locus or gene.

**flexible tissues** Nonhierarchical cell populations in which function and proliferation take place in the same cells.

**flow cytometry** Analysis of cell suspensions in which a dilute stream of cells is passed through a laser beam. DNA content and other properties are measured by light scattering and fluorescence following staining with dyes or labeled antibodies.

**founder effect** A form of genetic drift. The establishment of a population by a small number of individuals whose genotypes carry only a fraction of the different kinds of alleles **in** the parental population.

**fractionation** The daily dose of radiation based on the total dose divided into a particular number of daily treatments.

**fragile site** A heritable gap or nonstaining region of a chromosome that can be induced to generate chromosome breaks.

**frameshift mutation** A mutational event leading to the insertion of one or more base pairs in a gene, shifting the codon reading frame in all codons following the mutational site.

**free radical** A fragment of an atom or molecule containing an unpaired electron, therefore very reactive.

**fusion** Thermonuclear fusion occurs if two or more light nuclei coalesce to form a heavier nucleus with the release of energy.

**gamete** A specialized reproductive cell with a haploid number of chromosomes.

**gamma-rays (γ)** High-energy, short-wavelength electromagnetic radiation.  $\gamma$ -Radiation frequently accompanies  $\alpha$ - and  $\beta$ -emissions and always accompanies fission.  $\gamma$ -Rays are very penetrating and are stopped best or shielded against by dense materials, such as lead or depleted uranium.  $\gamma$ -Rays are indistinguishable from x-rays except for their source:  $\gamma$ -Rays originate inside the nucleus; x-rays from outside.

**gastrointestinal (GI)** Having to do with the digestive tract, which includes the mouth, esophagus, stomach, and intestines.

**gene** The fundamental physical unit of heredity whose existence can be confirmed by allelic variants and that occupies a specific chromosomal locus. A DNA sequence coding for a single polypeptide.

**gene amplification** The presence of multiple copies of a gene. This is one mechanism by which protooncogenes are activated to **result** in neoplasia.

**gene expression** The process of producing a protein from its DNA- and mRNA-coding sequences.

**gene mutation** See **point mutation**.

**gene pool** The total of all genes possessed by reproductive members of a population.

**genetic burden** Average number of recessive lethal genes carried in the heterozygous condition by an individual in a population. Also called *genetic load*.

**genetic code** The nucleotide triplets that code for the 20 amino acids or for chain initiation or termination.

**genetic counseling** Analysis of risk for genetic defects in a family and the presentation of options available to avoid or ameliorate possible risks.

**genetic drift** Random variation in gene frequency from generation to generation. Most often observed in small populations.

**genetic effects** Changes **in** reproductive cells that may result in abnormal offspring of persons or animals. Now called *heritable effects*.

**genetic effects of radiation** Radiation effects that can be transferred from parent to offspring. Any radiation-caused changes in the genetic material of sex cells. Now called *hereditary effects*.

**genetic polymorphism** The stable coexistence of two or more discontinuous genotypes in a population. If the frequencies of two alleles are earned to an equilibrium, the condition is called *balanced polymorphism*.

**genetically significant dose** The dose that, if given to every member of a population,

should produce the same hereditary harm as the actual doses received by the individuals. Expressed in sieverts, this dose takes into account the childbearing potential of those receiving the dose.

**genetics** The branch of biology that deals with heredity and the expression of inherited traits.

**genome** The genetic complement contained in the chromosomes of a given organism.

**genomic library** A library composed of fragments of genomic DNA.

**genotype** The specific allelic or genetic constitution of an organism; often, the allelic composition of one or a limited number of genes under investigation.

**GeV** One billion electron volts. Also written *BeV*.

**GI** gastrointestinal.

**giga** A prefix that multiplies a basic unit by one billion ( $10^9$ ).

**gonads** The ovaries or testes.

**grade** In reference to tumors, the aggressiveness of the cell type, from very low aggressiveness with slow growth pattern to very aggressive with rapid spread. Tumor grading classifications vary according to type of tumor.

**graft versus host disease (GVHD)** In transplants, reaction by immunologically competent cells of the donor against the antigens present on the cells of the host. In human bone-marrow transplants, often a fatal condition.

**granulocyte-colony stimulating factor (G-CSF)** Cytokine which stimulates differentiation of progenitors into granulocytes.

**granulocyte macrophage colony stimulating factor (GM-CSF)** Cytokine that stimulates differentiation of progenitors into granulocytes, macrophages, and eosinophils.

**gray (Gy)** The special name for the SI unit of absorbed dose, kerma, and specific energy imparted equal to 1 J/kg. The previous unit of absorbed dose, rad, has been replaced by the gray. One gray equals 100 rad.

**ground state** The state of a nucleus, an atom, or a molecule at its lowest (normal) energy level.

**growth delay** Extra time required for an irradiated tumor to reach a given size, compared with an unirradiated control.

**growth factors** Nonhormonal polypeptides involved in the development, growth, differentiation, and maintenance of tissues.

**growth fraction** Proportion of viable cells in active cell division.

**growth hormone (somatotropin) (GH)** Secreted by the anterior pituitary gland, acts mainly on the growth of bone and muscles. Can be secreted by lymphocytes in response to phorbol ester treatment, and may be involved in lymphocyte growth.

**half-life (Un)** The time taken for the activity of a radionuclide to decay to half its initial value.

**half-life, biologic** The time required for a biologic system, such as a human or an animal, to eliminate by natural processes half the amount of a substance, such as a radioactive material, that has entered it.

**haploid** Having only one of each type of chromosome, as is usually the case in gametes (oocytes and spermatozoa).

**heat shock** A transient response following exposure of cells or organisms to elevated temperatures. The response involves activation of a small number of loci, inactivation of previously active loci, and selective translation of heat shock mRNA. Appears to be a nearly universal phenomenon observed in organisms ranging from bacteria to humans.

**heavy water (D<sub>2</sub>O)** Water containing significantly more than the natural proportion (1 in 6,500) of heavy hydrogen (deuterium) atoms to ordinary hydrogen atoms. Heavy water is a moderator in some nuclear reactors because it slows down neutrons effectively and also has a low cross-section for absorption of neutrons.

**helicase** An enzyme that participates in DNA replication by unwinding the double helix near the replication fork.

**hematocrit** The percentage of blood made up of red blood cells.

**hematology** The study of blood and its disorders.

**hemizygous** Conditions in which a gene is present in a single dose. Usually applied to genes on the X chromosome in heterogametic males.

**hemoglobin (Hb)** An iron-containing, conjugated respiratory protein occurring chiefly in the red blood cells of vertebrates. Carries oxygen to the tissues.

**hemophilia** A sex-linked trait in humans associated with defective blood-clotting mechanisms.

**heredity** Transmission of traits from one generation to another.

**heritability** A measure of the degree to which observed phenotypic differences for a trait are genetic.

**heterochromatin** The heavily staining, late-replicating regions of chromosomes that are condensed in interphase. Thought to be devoid of structural genes.

**heterozygote** An individual with different alleles at one or more loci. Such individuals produce unlike gametes and therefore do not breed true.

**hierarchical tissues** Cell populations comprising a lineage of stem cells, proliferating cells, and mature cells. The mature cells do not divide.

**high dose rate (HDR) remote brachytherapy** A type of internal radiation in which each treatment is given in a few minutes with the radioactive source in place. The source of radioactivity is removed between treatments.

**histones** Proteins complexed with DNA in the nucleus. They are rich in the basic amino acids arginine and lysine and function in the coiling of DNA to form nucleosomes.

**HLA** Cell-surface proteins, produced by histocompatibility loci, which are involved in the acceptance or rejection of tissue and organ grafts and transplants.

**homogeneously staining region (hsr)** Segment of mammalian chromosomes that stains lightly with Giemsa following exposure of cells to a selective agent. These regions arise in conjunction with gene amplification and are regarded as the structural locus for the amplified gene.

**homozygote** An individual with identical alleles at one or more loci. Such individuals produce identical gametes and therefore breed true.

**hormones** Factors synthesized in endocrine glands that if released act to regulate and modulate the functions of multicellular organisms.

**hybrid** The offspring of two parents differing in at least one genetic characteristic.

**hybridization** The hydrogen bonding of complementary DNA or RNA sequences to form a duplex molecule.

**hybridoma** A somatic cell hybrid produced by the fusion of an antibody-producing cell and a cancer cell, specifically a myeloma. The cancer cell contributes the ability to divide indefinitely, and the antibody cell confers the ability to synthesize large amounts of a single antibody.

**hydrogen bond** An electrostatic attraction between a hydrogen atom bonded to a strongly electronegative atom such as oxygen or nitrogen and another atom that is electronegative or contains an unshared electron pair.

**hyperbaric oxygen (HBO)** The use of high oxygen pressures (two or three atmospheres) to enhance oxygen availability in radiotherapy.

**hyperdiploid** Additional chromosomes; the modal number is 47 or less.

**hyperfractionated radiation** Division of the total dose of radiation into smaller doses usually given more than once a day.

**hyperthermia** The use of heat treatments in excess of 42°C to treat cancer.

**hypodiploid** Loss of chromosomes with modal number 45 or less.

**hypofractionation** The use of dose fractions substantially larger than the conventional level of ~2 Gy.

**hypopharynx** Part of the lower throat beside and behind the larynx (voice box).

**hypoxia** Low oxygen tension; usually the very low levels that are required to make cells maximally radioresistant. Sometimes used to mean "anoxia" (literally, the complete absence of oxygen).

**identical twins** See **monozygotic twins**.

**IFNa** Cytokine produced in response to viral infection, protects cells from viruses, causes growth arrest of normal and tumor cells.

**IFNp** Cytokine produced in response to viral infection, protects cells from viruses, causes growth arrest of normal and tumor cells.

**IFNy** Cytokine produced in response to viral infection, activates macrophages, protects cells from viruses, causes growth arrest of normal and tumor cells.

**IL-1** Cytokine involved in the regulation of immune and inflammatory responses. Can be induced by many cytokines, UVB, and many other stimuli.

**IL-2** Cytokine that stimulates growth of T cells. Also stimulates B-cell growth and differentiation, generation of lymphokine-activated killer cells, activation of macrophages, and production of other cytokines.

**IL-3** Cytokine that induces the proliferation of hematopoietic cells, particularly erythroid and myeloid cells. Produced by activated T cells and mast cells.

**IL-4** Cytokine that has general effects on hematopoietic cells, including the activation, growth, and differentiation of B cells. Also induces growth of mast cells and T cells.

**IL-5** Cytokine that induces eosinophil differentiation, as well as B-cell activation, growth, and differentiation.

**IL-6** Cytokine that regulates B-cell differentiation, T-cell activation, killer-cell induction, and other physiologic responses. Induced by cytokines, ultraviolet irradiation, and other stimuli. Many effects similar to IL-1 and TNF.

**IL-7** Cytokine that promotes the growth of B and T cell progenitors.

**IL-8** One of an extended family of cytokines that act as chemoattractants for neutrophils, T cells, and basophils.

**IL-9** Cytokine that stimulates proliferation of T cells and enhances mast cell activity and growth.

**IL-10** Cytokine that enhances IL-2 and IL-4 proliferative response of T cells. Also acts

to inhibit cytokine production by a variety of different cells.

**IL-11** Cytokine that together with other cytokines stimulates the growth of a variety of hematopoietic progenitors.

**IL-12** Cytokine that promotes growth of T and natural killer cells and enhances cytotoxic T cell responses.

**immune system** The body's defense system that protects it from foreign substances such as bacteria and viruses that are harmful to it.

**immunoglobulin** The class of serum proteins having the properties of antibodies.

**implant** A quantity of radioactive material placed in or near a cancer.

**inbreeding** Mating between closely related organisms.

**incomplete repair** Increased damage from fractionated radiotherapy if the time interval between doses is too short to allow complete recovery of sublethal damage.

**indirect action** Damage to DNA by free radicals formed through the ionization of nearby water molecules.

**inducible response** A response to irradiation that is modified by a small dose of radiation given shortly before.

**infusion** Slow or prolonged intravenous delivery of a drug or fluid.

**initial slope** The steepness of the initial part of theoxic cell-survival curve, sometimes indicated by the **surviving fraction** at 2 Gy.

**initiating agent** Something that causes initial "latent" damage to the DNA. The cell requires more damage from a second "promoting agent" before the damage is expressed as cancer. Radiation usually is considered an initiating agent.

**initiation codon** The mRNA sequence AUG, which codes for methionine and initiates translation.

**in situ hybridization** A technique for the cytologic localization of DNA sequences complementary to a given nucleic acid or polynucleotide.

**intercalating agent** A compound that inserts between bases in a DNA molecule, disrupting the alignment and pairing of

bases **in** the complementary strands (e.g., acridine dyes).

**interferon** One of a family of proteins that act to inhibit viral replication in higher organisms. Some interferons may have anti-cancer properties.

**interphase** That portion of the cell cycle between divisions.

**interphase death** The death of irradiated cells before they reach mitosis.

**interstitial implant** The placement of fine tubes in a grid-like pattern through tissues containing a cancer; these tubes are filled later with radioactive sources for brachytherapy.

**intracavitory implant** The placement of a small tube within a body cavity, such as the bronchus or vagina; this tube later is filled with radioactive sources for brachytherapy.

**intraoperative radiation** A type of radiation used to deliver a large dose of radiation therapy to the tumor bed and surrounding tissue at the time of surgery.

**intravenous (IV)** Into a vein.

**intron** A portion of DNA between coding regions in a gene which is transcribed, but which does not appear in the mRNA product.

**inverse square law** A physical law stating that the intensity of x- or y-radiation from a point source emitting uniformly in all directions is inversely proportional to the square of the distance from the source. Example: A point source that produces 10 Gy/h at 1 m produces 2.5 Gy/h at 2 m.

**inversion** Two breaks occur in the same chromosome with rotation of the intervening segment. If both the breaks are on the same side of the centromere, it is called a paracentric inversion. If they are on opposite sides, it is called a **pericentric inversion**.

**in vitro** Literally, in glass; outside the living organism; occurring in an artificial environment.

**in vivo** Literally, in the living; occurring within the living body of an organism.

**ion** An electrically charged atom or group of atoms.

**ion pair** A closely associated positive ion and negative ion (usually an electron) hav-

ing charges of the same magnitude and formed from a neutral atom or molecule by radiation.

**ionization** The process of adding one or more electrons to, or removing one or more electrons from, atoms or molecules, thereby creating ions. High temperatures, electrical discharges, or nuclear radiations can cause ionization.

**ionization chamber** A device for detection of ionizing radiation or for measurement of radiation dose and dose rate.

**ionizing radiation** Any radiation displacing electrons from atoms or molecules, thereby producing ions. Examples of ionizing radiation are alpha ( $\alpha$ ), beta ( $\beta$ ), and gamma ( $\gamma$ ) radiation and short-wave ultraviolet light. Ionizing radiation may produce severe skin or tissue damage. See **radiation burn, radiation illness**.

**ipsilateral** On the same side of the body (opposite of contralateral).

**irradiation** Exposure to radiation, as in a nuclear reactor.

**isobar** Atoms having the same number of nucleons but different numbers of protons and neutrons.

**isochromosome** A chromosome that consists of identical copies of one chromosome arm with loss of the other arm. Thus, an isochromosome for the long arm of chromosome 17 (i[17][ql0]) contains two copies of the long arm (separated by the centromere) with loss of the short arm of the chromosome.

**isoeffect plots** Graphs of the total dose for a given effect (e.g., ED<sub>50</sub>) plotted, for instance, against dose per fraction or dose rate.

**isomer** Atoms having the same number of protons and neutrons but a different nuclear-energy state.

**isotopes** Forms of a chemical element that have the same number of protons and electrons but differ in the number of neutrons contained in the atomic nucleus. Unstable isotopes undergo a transition to a more stable form with the release of radioactivity.

**isotropic** Equal intensity in all directions.

**Karnofsky score** A measure of the patient's overall physical health following treatment, judged by his or her level of activity.

**karyotype** Arrangement of chromosomes from a particular cell according to a well-established system such that the largest chromosomes are first and the smallest ones are last. Normal female karyotype is 46,XX; normal male karyotype is 46,XY.

**kerma** The sum of the initial kinetic energies of all the charged ionizing particles liberated by uncharged ionizing particles per unit mass of a specified material. Kerma is measured in the same unit as absorbed dose. The SI unit of kerma is joule per kilogram, and its special name is gray (Gy). Kerma can be quoted for any specified material at a point in free space or in an absorbing medium.

**kilobase (kb)** A unit of length consisting of 1,000 nucleotides.

**kilovolt (kV)** A unit of electric potential difference equal to 1,000 V.

**labeling index (LI)** Proportion or percentage of cells in the population (S phase) that take up tritiated thymidine or other precursors, such as bromodeoxyuridine; *i.e.*, the proportion synthesizing DNA.

**larynx** Part of the throat used for speaking; often called the voice box or Adam's apple.

**late responses** Radiation-induced normal-tissue damage that **in** humans is expressed months to years after exposure. Generally results from damage to connective tissue cells, **a/p** ratio tends to be small (< 5 Gy).

**latency period** The time between an injury occurring and the effects of the injury expressing themselves as disease.

**LD50/30** Radiation dose to produce lethality in 50% of animals by 30 days, similarly LD50/7, *etc.*

**leader sequence** That portion of an mRNA molecule from the 5' end to the beginning codon; may contain regulatory or ribosome binding sites.

**leading strand** During DNA replication, the strand synthesized continuously 5' to 3' toward the replication fork.

**leakage radiation** All radiation coming from within the source assembly except for the useful beam. (Leakage radiation includes the portion of the radiation coming directly from the source and not absorbed by the source assembly, as well as the scattered radiation produced within the source assembly.)

**LET** Linear energy transfer.

**lethal dose** A dose of ionizing radiation sufficient to cause death. Median lethal dose (MLD or LD50) is the dose required to kill, within a specified period of time, half the individuals in a large group of organisms similarly exposed. The *LD<sub>50/tio</sub>* for humans is about 4 Gy (400 rad).

**lethal gene** A gene whose expression results in death.

**leukemia** A malignant cancer of the blood-forming tissues (bone marrow or lymph nodes), generally characterized by an over-production of white blood cells.

**leukocyte** White blood cell.

**library** A collection of cells (usually bacteria or yeast) that have been transformed with recombinant vectors carrying DNA inserts from a single species.

**lifetime risk** The risk of dying of some particular cause over the whole of a person's life.

**ligase** An enzyme that catalyzes a reaction that links two DNA molecules by the formation of a phosphodiester bond.

**ligation** The process of joining two or more DNA fragments.

**linear accelerator** A machine creating high-energy radiation to treat cancers, using electricity to form a stream of fast-moving subatomic particles. Also called *megavoltage linear accelerator* or a *linac*.

**linear energy transfer (LET)** The rate of energy loss along the track of an ionizing particle. Usually expressed in keV/itm.

**linear-quadratic (LQ) model** Model in which the effect (E) is a linear-quadratic function of dose (d):  $E = ad + (3d^2)$ . For cell survival:  $S = \exp(-ad - (3d^2))$ .

**linkage** Condition in which two or more non-allelic genes tend to be **inherited** together. Linked genes have their loci along the same

chromosome, do not assort independently, but can be separated by crossing over.

**local invasion** The spread of cancer from an original site to the surrounding tissues.

**localized tumors** Tumors that are contained in one particular site and have not spread.

**log-phase culture** A cell culture growing exponentially.

**long terminal repeat (LTR)** Sequence of several hundred base pairs found at the ends of retroviral DNAs.

**lymph node** A collection of lymphocytes within a capsule and connected to other lymph nodes by fine lymphatic vessels; a common site for certain cancer cells to grow after traveling along lymphatic vessels.

**lymphatic system** A network of fine lymphatic vessels that collects tissue fluids from all over the body and returns these fluids to the blood. Accumulations of lymphocytes, called *lymph nodes*, are situated along the course of lymphatic vessels.

**lymphocyte** A type of white blood cell that helps protect the body against invading organisms by producing antibodies and regulating the immune-system response.

**lymphoma** A type of cancer beginning in an altered lymphocyte. There are two broad categories of lymphomas, Hodgkin's disease and non-Hodgkin's lymphoma.

**lysis** The disintegration of a cell brought about by the rupture of its membrane.

**macrophage** A type of white blood cell assisting in the body's fight against bacteria and infection by engulfing and destroying invading organisms.

**macrophage colony stimulating factor (M-CSF)** Cytokine that stimulates formation of macrophages from pluripotent hematopoietic cells.

**magnetic resonance imaging (MRI)** A method of taking pictures of body tissue using magnetic fields.

**mammogram** An x-ray of the breast used to detect cancer, sometimes before it can be detected by palpation. Women older than 50 years are advised to have a mammogram every year; women in their forties, every 2 years.

**mass number (A)** The sum of the neutrons and protons in a nucleus. It is the nearest whole number to an isotope's atomic weight. For instance, the mass number of the uranium-235 isotope is 235. Compare **atomic number**.

**mean** Arithmetic average.

**median** The value in a group of numbers below and above which there are an equal number of data points or measurements.

**medical oncologist** A doctor specializing in using chemotherapy to treat cancer.

**mega** A prefix that multiplies a basic unit by one million ( $10^6$ ).

**meiosis** The process in gametogenesis or sporogenesis during which one replication of the chromosomes is followed by two nuclear divisions to produce four haploid cells.

**melanoma** A type of cancer that begins in the pigment-containing cells of a skin mole or the lining of the eye.

**meningioma** A type of brain tumor that is relatively common and usually benign.

**messenger RNA (mRNA)** The class of RNA molecules that copies the genetic information from DNA, in the nucleus, and carries it to ribosomes, in the cytoplasm.

**metacentric chromosome** A chromosome with a centrally located centromere, producing chromosome arms of equal lengths.

**metaphase** The stage of cell division in which the condensed chromosomes lie in a central plane between the two poles of the cell, and in which the chromosomes become attached to the spindle fibers.

**metastasis** Cancer cells breaking away from their original site and spread to other parts of the body.

**metastatic cancer** An advanced stage of cancer in which cells from the original (primary) site have spread (metastasized) to other organs.

**MeV** One million ( $10^6$ ) electron volts.

**micro-(ji)** A prefix that divides a basic unit by one million ( $1CT^6$ ).

**microinjection** Introducing DNA into a cell using a fine microcapillary pipet.

**micrometer (jim)** A unit of length equal to  $1 \times 10^{-6}$  meter. Previously called a *micron*.

**micron** See **micrometer**.

**migration coefficient** An expression of the proportion of migrant genes entering the population per generation.

**millimeter (mm)** A unit of length equal to  $1 \times 10^{-3}$  m.

**minimal medium** A medium containing only those nutrients that support the growth and reproduction of wild-type strains of an organism.

**missense mutation** A mutation that alters a codon to that of another amino acid, causing an altered translation product to be made.

**mitochondrion** Found in the cells of eukaryotes; a cytoplasmic, self-reproducing organelle that is the site of ATP synthesis.

**mitogen** A substance that stimulates mitosis in nondividing cells; *e.g.*, phytohemagglutinin.

**mitogen-activated protein (MAP) kinase** A family of two protein kinases of 42 and 44 kDa (ERK1 and ERK2) and 38 kDa that act to induce certain early-response genes.

**mitosis** Process of nuclear division in which chromosomes move along a spindle resulting in two new nuclei with the same number of chromosomes as the original nucleus.

**mitotic death** Cell death associated with a postirradiation mitosis.

**mitotic delay** Delay of entry into mitosis, or accumulation in G<sub>1</sub>, as a result of treatment.

**mitotic index (MI)** Proportion or percentage of cells in mitosis at any given time.

**molecule** A group of atoms held together by chemical forces. The atoms of the molecule may be identical, as in H<sub>2</sub>, and S<sub>8</sub>, or different, as in H<sub>2</sub>O and CO<sub>2</sub>. A molecule is the smallest unit of matter that can exist by itself and retain all its chemical properties.

**monoclonal antibodies** Immunoglobulin molecules of single-epitope specificity.

**monosomic** An aneuploid condition in which one member of a chromosome pair is missing; having a chromosome number of 2n - 1.

**monozygotic twins** Twins produced from a single fertilization event; the first division of the zygote produces two cells, each of which develops into an embryo. Also known as identical twins.

**morbidity** Sickness, side effects, and symptoms of a treatment or disease.

**mRNA (messenger RNA)** An RNA molecule transcribed from DNA and translated into the amino acid sequence of a polypeptide.

**mtDNA** Mitochondrial DNA.

**mucositis** Inflammation of the lining of areas such as the mouth.

**multitarget equation** Model that assumes the presence of a number of critical targets in a cell, all of which require inactivation to kill the cell. Survival is given by:  $S = 1 - [1 - \exp(-D/D_0)]^n$ .

**mutagen** Any agent that causes an increase in the rate of mutation.

**mutant** A cell or organism carrying an altered or mutant gene.

**mutation** A relatively stable change in the DNA of the cell nucleus. Mutations in the germ cells of the body (ova and sperm) may lead to inherited effects in the offspring. Mutations in the somatic cells of the body may lead to effects in the individual, *e.g.*, cancer.

**mutation rate** The frequency with which mutations take place at a given locus or in a population.

**myc** A nuclear oncogene involved in immortalizing cells.

**nano (n)** A prefix that divides a basic unit by one billion ( $10^{-9}$ ).

**nanometer (nm)** A unit of length equal to  $1 \times 10^{-9}$  m.

**nasopharynx** Part of the breathing passage behind the nasal cavity.

**natural radioactivity** See **background radiation**.

**natural selection** Differential reproduction of some members of a species resulting from variable fitness conferred by genotypic differences.

**natural uranium** Uranium as found in nature, containing 0.7% of the isotope <sup>235</sup>U, 99.3% of <sup>238</sup>U, and a trace of <sup>234</sup>U.

**neutrino** (v) An electrically neutral elementary particle with a negligible mass. It interacts very weakly with matter and hence is difficult to detect. It is produced in many nuclear reactions, for example, in beta decay, and has high penetrating power. Neutrinos from the sun usually pass right through the earth.

**neutron (n)** An uncharged elementary particle with a mass slightly greater than that of the proton and found in the nucleus of every atom heavier than hydrogen. A free neutron is unstable and decays with a half-life of about 13 minutes into an electron, a proton, and a neutrino. Neutrons sustain the fission chain reaction in a nuclear reactor.

**nondisjunction** An accident of cell division in which the homologous chromosomes (in meiosis) or the sister chromatids (in mitosis) fail to separate and migrate to opposite poles; responsible for defects such as monosomy and trisomy.

**nonsense mutation** A mutation that alters a codon to one that encodes no amino acid; i.e., UAG (amber codon), UAA (ochre codon), or UGA (opal codon). Leads to premature termination during the translation of mRNA.

**nonstochastic effect** An effect the severity of which increases with increasing dose, after a threshold region. Now called **deterministic effect**.

**normal distribution** A probability function that approximates the distribution of random variables. The normal curve, also known as a *Gaussian* or *bell-shaped curve*, is the graphic display of the normal distribution.

**Northern blotting** A procedure in which RNA fragments are transferred from an agarose gel to a nitrocellulose filter, from which the RNA then is hybridized to a radioactive probe.

**NSD** Nominal standard dose in the Ellis formula.

**nuclear reactor** A structure in which nuclear fission may be sustained in a self-supporting chain reaction. In thermal reac-

tors the fission is produced by fission neutrons, and in fast reactors by fast neutrons.

**nuclease** An enzyme that breaks bonds in nucleic acid molecules.

**nucleic acid** A class of organic acids that play a role in protein synthesis, in the transmission of hereditary traits, and in the control of cellular activities.

**nucleon** Proton or neutron.

**nucleoside** A purine or pyrimidine base covalently linked to a ribose or deoxyribose sugar molecule.

**nucleotide** A nucleoside covalently linked to a phosphate group. Nucleotides are the basic building blocks of nucleic acids. The nucleotides commonly found in DNA are deoxyadenylic acid, deoxycytidylic acid, deoxyguanylic acid, and deoxythymidylic acid. The nucleotides in RNA are adenylic acid, cytidylic acid, guanylic acid, and uridylic acid.

**nucleotide pair** The pair of nucleotides (A and T or G and C) in opposite strands of the DNA molecule that are hydrogen-bonded to each other.

**nucleus (of an atom)** The small, positively charged core of an atom. It is only about 1/10,000 the diameter of the atom but contains nearly all the atom's mass. All nuclei contain both protons and neutrons, except the nucleus of ordinary hydrogen, which consists of a single proton.

**nucleus (of a cell)** The membrane-bound cytoplasmic organelle of eukaryotic cells that contains the chromosomes and nucleolus.

**nuclide** A general term applicable to all atomic forms of the elements. The term often is used incorrectly as a synonym for *isotope*, which properly has a more limited definition. Whereas isotopes are the various forms of a single element (hence are a family of nuclides) and all have the same atomic number and number of protons, nuclides comprise all the isotopic forms of all the elements. Nuclides are distinguished by their atomic number, atomic mass, and energy state.

**occupationally exposed** Exposed to radiation as a direct result of occupational duties.

**oncogene** A gene that contributes to cancer formation when mutated or inappropriately expressed.

**oncogenic** A factor or agent with the potential to cause cancer (same as carcinogenic).

**oncologist** A physician specializing in the study and treatment of cancer.

**oncology** The study of cancer.

**I 4-3-3** A protein that interacts with *raf* to promote translocation to the cell membrane, in which *raf* in turn, interacts with *ras*.

**open reading frame** A long DNA sequence, uninterrupted by a stop codon, that encodes part or all of a protein.

**oxygen enhancement ratio (OER)** The ratio of the radiation dose given under anoxic conditions to produce a given effect relative to the radiation dose given under fully oxygenated conditions to produce the same effect.

**p15** Gi inhibitor induced in epithelial cells by TGF $\beta$ . Inhibits cyclin D1-Cdk4 and cyclin D1-Cdk6 complexes.

**p16** Gi inhibitor of epithelial cells. Inhibits cyclin D1-Cdk4 and cyclin D1-Cdk6 complexes. Gene is deleted in familial melanomas and other tumor types.

**p21 (WAF1)** Inhibitor of Cdc2, Cdk4, and Cdk6. Induced through p53 pathway.

**p27** Cell-cycle inhibitor induced in epithelial cells of TGF $\beta$ . Inhibits cyclin E-Cdk2 complex.

**p53** Considered the guardian of the genome. Mediates cellular responses to DNA damaging agents such as ionizing radiation at the Gi checkpoint. Induces p21. p53 gene on chromosome 17 is mutated in colon, breast, esophageal, and a variety of other human cancers. Binds DNA; can act as transcription factor.

**palindrome** A word, number, verse, or sentence that reads the same backward or forward. In nucleic acids, a sequence in which the base pairs read the same on complementary strands (5' -> 3'). For example: 5' GAATTC 3', 3' CTTAAG 5'. These often occur as sites for restriction endonuclease recognition and cutting.

**palliative care** Treatment to relieve, rather than cure, symptoms caused by cancer. Palliative care can help people live more comfortably.

**palpate** To examine by carefully feeling with the fingers.

**paracentric inversion** A chromosomal inversion that does not include the centromere.

**parent** Radioactive atom that disintegrates to give a different atom, its progeny.

**particle** A minute constituent of matter, generally one with a measurable mass. The primary particles involved in radioactivity are  $\alpha$ -particles, ( $\beta$ -particles, neutrons, and protons.

**pathologist** A specialist who attempts to describe the nature of a disease by analyzing samples obtained from tissues, organs, or body fluids.

**pathology** The study of diseased tissues, both by gross and by microscopic examination of tissues removed during surgery and postmortem.

**pedigree** In human genetics, a diagram showing the ancestral relationships of a given genotype manifest in at least some of a specific mutant phenotype associated with a trait.

**penetrance** The frequency (expressed as a percentage) with which individuals of a given genotype manifest at least some degree of a specific mutant phenotype associated with a trait.

**peptide bond** The covalent bond between the amino group of one amino acid and the carboxyl group of another amino acid.

**pericentric inversion** A chromosomal inversion that involves both arms of the chromosome and thus involves the centromere.

**phage** See bacteriophage.

**pharynx** Medical term for the throat from the nasal and oral cavities above to the larynx and esophagus below.

**phosphodiester bond** In nucleic acids, the covalent bond between a phosphate group and adjacent nucleotides, extending from the 5' carbon of one pentose (ribose or deoxyribose) to the 3' carbon of the pentose

in the neighboring nucleotide. Phosphodiester bonds form the backbone of nucleic acid molecules.

**photodynamic therapy** Cancer treatment using light to activate a photosensitizing agent, thereby releasing cytotoxic free radicals.

**photoelectric effect** Absorption of an x-ray by ionization.

**photon** The carrier of a quantum of electromagnetic energy. Photons have an effective momentum but no mass or electric charge.

**pico-(p)** A prefix that divides a basic unit by one trillion ( $10^{-12}$ ). Same as *micromicro-*.

**picocuries per liter (pCi/L)** A unit of measurement of the activity concentration of a radioactive material; measures, for example, how many radioactive disintegrations of radon occur every second **in** a liter of air.

**plaque** A clear spot on a lawn of bacteria or cultured cells on which cells have been lysed by viral infection and replication.

**plasmid** An extrachromosomal, circular DNA molecule (often carrying genetic information) that replicates independently of the host chromosome.

**plateau-phase cultures** Cell cultures grown to confluence so that proliferation is markedly reduced (^stationary phase).

**platelets** Special blood cells that help stop bleeding.

**platelet-derived growth factor (PDGF)** Also, *mesenchymal-derived cells*; also acts on some epithelial and endothelial cells. Induces growth fibroblasts and is involved in wound healing.

**plating efficiency** The proportion or percentage of *in vitro* plated cells that form colonies.

**pleiotropy** Condition in which a single mutation simultaneously affects several characters.

**ploidy** Relates to the number of sets of chromosomes in a cell. Diploid cells have two sets of chromosomes, a chromosome complement twice that is found in the gametes. Tetraploid cells have four sets of chromosomes.

**point mutation** A mutation that can be mapped to a single locus. At the molecular level, a mutation that results in the substitution of one nucleotide for another.

**polar body** A cell produced at either the first or second meiotic division in females, which contains almost no cytoplasm as a result of unequal cytokinesis.

**polyacrylamide gel electrophoresis** Electrophoresis through a matrix composed of a synthetic polymer, used to separate small DNA or RNA molecules (up to 1,000 nucleotides) or proteins.

**polyclonal antibodies** A mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different epitope.

**polymerase** An enzyme that catalyzes the addition of multiple sub-units to a substrate molecule.

**polymerase chain reaction (PCR)** A procedure that enzymatically amplifies a DNA sequence through repeated replication by DNA polymerase.

**polymorphism** The existence of two or more discontinuous, segregating phenotypes in a population.

**polypeptide** A molecule made up of amino acids joined by covalent peptide bonds. This term is used to denote the amino acid chain before it assumes its functional three-dimensional configuration.

**polyploid** A cell or individual having more than two sets of chromosomes.

**population** A local group of individuals belonging to the same species, which are actually or potentially interbreeding.

**positron ( $(3^+)$ )** An elementary particle with the mass of an electron but charged positively. It is the "antielectron." It is emitted in some radioactive disintegrations and is formed by the interaction of high-energy  $\gamma$ -rays with matter.

**potential doubling time ( $T_{pot}$ )** Tumor doubling time, taking into account the cell cycle time and the growth fraction, but ignoring cell loss.

**potentially lethal damage (PLD)** Cellular damage that is repaired during the interval

between treatment and assay, especially under suboptimal growth conditions.

**PRb** A protein of ~110 kD that regulates cell-cycle progression through G1. Phosphorylated by cyclin D-Cdk complexes to release Gi transcription factors. Inactivated in hereditary retinoblastoma and sporadic tumors of the bone, breast, esophagus, and other tissues.

**precursor** A precursor of an atom in a radioactive decay chain is a member of the decay chain that occurs before the atom in question.

**primary tumor** The place in which the cancer originates, which is referred to regardless of the site of its eventual spread. Thus, prostate cancer that spreads to the bone is still prostate cancer and is not referred to as bone cancer.

**primer** A short DNA or RNA fragment annealed to single-stranded DNA.

**primordial** Existing at the beginning of the universe, or at the beginning of the earth.

**priori** An infectious pathogenic agent devoid of nucleic acid and composed mainly of a protein. PrR with a molecular weight of 27,000 to 30,000 daltons. Prions are known to cause scrapie, a degenerative neurologic disease in sheep, and are thought to cause similar diseases in humans, such as kuru and **Creutzfeldt-Jakob** disease.

**probability** Ratio of the frequency of a given event to the frequency of all possible events.

**probe** A single-stranded DNA (or RNA) that has been labeled radioactively and is used to identify complementary sequences.

**prodromal phase** Signs and symptoms in the first 48 hours following irradiation of the central nervous system.

**progeny** Formerly called a "daughter" in a radioactive decay chain.

**prognosis** The predicted or likely outcome.

**prokaryotes** Organisms lacking nuclear membranes, meiosis, and mitosis. Bacteria and blue-green algae are examples of prokaryotic organisms.

**promoting agent** Something that acts on earlier cellular damage caused by an initiat-

ing agent; can cause the earlier damage to be expressed as cancer. Tobacco smoke usually is considered a promoting agent.

**promotor site** Region having a regulatory function and to which RNA polymerase binds prior to the initiation of transcription.

**prophylactic** Preventive measure or medication.

**prostate** A gland at the base of the bladder in males for the production of seminal fluids. Cancer of this gland is common in elderly men.

**protein** A molecule composed of one or more polypeptides, each composed of **amino acids** covalently linked together.

**protein kinase C (PKC)** A family of protein kinases involved in mitogenic signalling. Activated by second messengers, including diacylglycerol and  $\text{Ca}^{2+}$  (some isoforms). Can be activated directly by the phorbol ester class of tumor promoters. Can induce early-response **genes** through *raf*.

**protocol** A standardized combination of therapies developed specifically for particular tumors.

**proton** An elementary particle that is a component of all nuclei with a single positive electric charge and a mass approximately 1,837 times that of the electron. The nucleus of an ordinary or light hydrogen atom. The atomic number of an atom is equal to the number of protons in its nucleus.

**purines** Organic bases with carbon and nitrogen atoms in two interlocking rings; components of nucleic acids and other biologically active substances.

**pyrimidines** Nitrogenous bases composed of a single ring of carbon and nitrogen atoms; components of nucleic acids.

**quality factor** The factor by which absorbed dose is to be multiplied to obtain a quantity that expresses on a common scale, for all ionizing radiations, the irradiation incurred by exposed persons. Largely replaced by radiation weighting factor.

**quasithreshold dose ( $D_q$ )** Point of extrapolation of the exponential portion of a multi-

target survival curve to the level of zero survival:  $D_q = D_0 \ln(n)$ .

**rad** The old unit of absorbed dose, equivalent to an energy absorption of  $10^{-2}$  J/kg. Superseded by the gray. See **absorbed dose**.

**radiation** The emission and propagation of energy through matter or space by means of electromagnetic disturbances that display both wave-like and particle-like behavior; in this context the "particles" are known as *photons*. Also, the energy so propagated. The term has been extended to include streams of fast-moving particles, such as  $\alpha$ - and  $\beta$ -particles, free neutrons, and cosmic radiation. Nuclear radiation is that emitted from atomic nuclei in various nuclear reactions, including alpha, beta, and gamma radiation and neutrons.

**radiation (ionizing)** Any electromagnetic or particulate radiation capable of producing ions, directly or indirectly, by interaction with matter. Examples are x-rays, photons, charged atomic particles and other ions, and neutrons.

**radiation absorbed dose** See **rad**.

**radiation accidents** Accidents resulting in the spread of radioactive material or in the exposure of individuals to radiation.

**radiation burn** Radiation damage to the skin. (3-Burns result from skin contact with or exposure to emitters of  $\beta$ -particles. See **beta-particle, ionizing radiation**.)

**radiation chemistry** The branch of chemistry that is concerned with the chemical effects, including decomposition, of energetic radiation or particles on matter.

**radiation dose** The amount of radiation absorbed by an irradiated object. This unit is the gray (Gy), defined to be 1 J/kg.

**radiation dosimetry** The measurement of the radiation delivered to a specific place or the amount of radiation that was absorbed there. See **dosimeter**.

**radiation illness** An acute organic disorder that follows exposure to relatively large doses of ionizing radiation. It is characterized by nausea, vomiting, diarrhea, blood cell changes, and in later stages by hemorrhage and loss of hair.

**radiation oncologist** A physician specializing in the treatment of tumors by radiation therapy.

**radiation protection** Legislation and regulations to protect the public and workers against radiation. Also, measures to reduce exposure to radiation.

**radiation quality** Relative penetrability of an x-ray beam determined by its average energy; usually measured by HVL (half value layer) or kVp (peak kilovoltage).

**radiation shielding** Reduction of radiation by interposing a shield of absorbing material between any radioactive source and a person, laboratory area, or radiation-sensitive device.

**radiation sterilization** Using radiation to make a plant or animal sterile. Also, radiation to kill all forms of life, especially bacteria, in food or surgical equipment.

**radiation therapy** Treatment of disease with any type of radiation. Often called *radiotherapy*.

**radiation warning symbol** An officially prescribed symbol, a magenta trefoil on a yellow background, that should be displayed whenever a radiation hazard exists.

**radioactive decay** See **decay, radioactive**.

**radioactive half-life** Time for a radioisotope to decay to one half its activity.

**radioactive isotope** One of the forms of an element, differing in atomic weight and possessing an unstable nucleus that emits ionizing radiation.

**radioactive series** A succession of nuclides, each in turn transforming by radioactive disintegration into the next nuclide until a stable nuclide is reached. The first member is called the *parent*, the intermediate members are called *progeny*, and the final, stable member is called the *end product*.

**radioactive tracer** A small quantity of a radioactive isotope, either with or without a carrier, used to follow biologic, chemical, or other processes by detection, determination, or localization of the radioactivity.

**radioactive waste** Unwanted radioactive materials in any form. Often categorized in

the nuclear power industry into low-level, intermediate-level, and high-level waste.

**radioactivity** A property of all unstable elements that regularly decay to an altered state by releasing energy in the form of photons ( $\gamma$ -rays) or particles (electrons,  $\alpha$ -particles, etc.).

**radiobiology** The body of knowledge and the study of the principles, the mechanisms, and the effects of ionizing radiation on living matter.

**radiogenic** Of radioactive origin; produced by radioactive transformation.

**radioisotope** A radioactive isotope. An unstable isotope of an element that decays or disintegrates spontaneously, emitting radiation. More than 1,300 natural and artificial radioisotopes have been identified.

**radiologist** A physician with special training in reading diagnostic x-rays and performing specialized x-ray procedures.

**radiology** The science that investigates all forms of ionizing radiation in the diagnosis and the treatment of disease.

**radionuclide** A radioactive nuclide.

**radioresistance** A relative resistance of cells, tissues, organs, or organisms to the harmful action of radiation.

**radioresponsiveness** A general term indicating the overall level of clinical response to radiotherapy.

**radiosensitivity** (1) A relative susceptibility of cells, tissues, organs, or organisms to the effects of radiation. (2) The radiation dose required to produce a defined level of cell inactivation. Usually indicated by the surviving fraction at 2 Gy (i.e., SF2) or by the parameters of the linear-quadratic or multitarget equations.

**radiosensitizer** In general, any agent that increases the sensitivity of cells to radiation. Most commonly applied to electron-affinic chemicals that mimic oxygen in fixing free-radical damage.

**radiotherapy** The treatment of disease with ionizing radiation. Often termed "radiation therapy."

**radium (Ra)** A radioactive metallic element with atomic number 88. As found in

nature, the most common isotope has an atomic weight of 226. It occurs in minute quantities associated with uranium in pitchblende, carnotite, and other minerals. The uranium decays to radium in a series of  $\alpha$ - and  $\beta$ -emissions. By virtue of being an  $\alpha$ - and  $\gamma$ -emitter, radium is used as a source of luminescence and as a radiation source in medicine and radiography.

**radon (Rn)** Colorless, odorless, naturally occurring radioactive gas. A radioactive element and the heaviest gas known. Its atomic number is 86 and its atomic weight varies from 200 to 226. It is the progeny of radium in the uranium radioactive series.

**radon daughter** Any atom that is below radon-222 in the uranium-decay chain; often specifically refers to polonium-218 and polonium-214, as these have the most biologic significance; now referred to as *radon progeny*.

**raf** A protein kinase that is activated by GTP-bound *ras*. Acts to transduce mitogenic signalling by phosphorylation of MAP kinases.

**ras** A family of 21-kD proteins (H-, K-, and N-ras) found to be activated by point mutations at codons 12, 13, and 61 in a variety of tumors. Involved in mitogenic signalling, coupling growth signals from growth factor receptors to *raf* activation, and downstream stimulation of early-response genes. Binds GTP in its activated state. Is found at the inner face of the cell membrane.

**RBE** Relative biologic effectiveness.

**reading frame** A series of triplet codons beginning from a specific nucleotide.

**reassortment** or **redistribution** Return towards a more even cell-age distribution, following the selective killing of cells in certain phases of the cell cycle.

**recessive** Term **describing** an allele that is not expressed in the heterozygous condition.

**recessive gene** The phenotype is expressed only if both copies of the gene are mutated or missing.

**reciprocal translocation** A chromosomal aberration in which nonhomologous chromosomes exchange parts.

**recombinant DNA** A DNA molecule formed by the joining of two heterologous molecules. Usually applied to DNA molecules produced by *in vitro* ligation of DNA from two different organisms.

**recovery** An increase in cell survival as a function of time during or after irradiation. See **repair**.

**recurrence** The return of a cancer after all detectable traces had been removed by primary therapy; recurrences may be local (near the primary site) or distant (metastatic).

**relapse** Recurrence of a disease following treatment.

**relative biologic effectiveness (RBE)** A factor used to compare the biologic effectiveness of different types of ionizing radiation. It is the inverse ratio of the amount of absorbed radiation required to produce a given effect to a standard (or reference) radiation required to produce the same effect.

**relative risk** Situation in which the risk of a disease resulting from some injury is expressed as some percentage increase of the normal rate of occurrence of that disease; in contrast to an absolute risk, in which the risk of a disease resulting from an injury does not depend on the normal rate of occurrence of that disease.

**rem** Old unit of equivalent or effective dose. It is the product of absorbed dose (in rads) and the radiation weighting factor. One rem is one hundredth of a sievert.

**remediation** The fixing of a home to reduce its indoor radon level.

**remission, complete** Condition in which no cancerous cells can be detected and the patient appears to be free of disease.

**remission, partial** Generally means that by all methods used to measure the existence of a tumor, there has been at least a 50% regression of the disease following treatment.

**remote brachytherapy** See **high dose rate remote brachytherapy**.

**reoxygenation** The process by which surviving hypoxic clonogenic cells become better oxygenated during the period after irradiation of a tumor.

**repair** Restoration of the integrity of damaged macromolecules.

**repair saturation** Explanation of the shoulder on cell-survival curves on the basis of the reduced effectiveness of repair after high radiation doses.

**reproductive death** The loss of the proliferative ability of a cell. Commonly restricted to those cells having an indefinite capacity to divide.

**reproductive integrity** Ability of cells to divide many times and thus be "clonogenic."

**resection** Surgical removal; in relation to cancer resection, the pathologist often indicates if the outer margins of the resection had no cancer cells present or were "negative."

**restriction endonuclease** Nuclease that recognizes specific nucleotide sequences in a DNA molecule, and cleaves or nicks the DNA at that site. Derived from a variety of microorganisms, those enzymes that cleave both strands of the DNA are used in the construction of recombinant DNA molecules.

**restriction fragment length polymorphism (RFLP)** Differences in nucleotide sequence between alleles that result in restriction fragments of varying lengths.

**retrovirus** A class of viruses whose genome consists of RNA and that utilizes the enzyme reverse transcriptase to copy its genome into a DNA intermediate, which integrates into the chromosomes of a host cell.

**reverse transcriptase** An enzyme that synthesizes a complementary DNA strand from an RNA template.

**reversion** A mutation that restores the wild-type phenotype.

**Rh factor** An antigenic system first described in the rhesus monkey. Recessive **r/r** individuals produce no Rh antigens and are Rh negative; **R/R** and **R/r individuals** have Rh antigens on the surface of their red blood cells and are classified as Rh positive.

**ribosomal RNA** See **rRNA**.

**RNA (ribonucleic acid)** An organic acid composed of repeating nucleotide units of adenine, guanine, cytosine, and uracil, whose ribose components are linked by phosphodiester bonds.

**RNA polymerase** An enzyme that catalyzes the formation of an RNA polynucleotide strand using the base sequence of a DNA molecule as a template.

**Robertsonian translocation** A form of chromosomal aberration that involves the fusion of long arms of acrocentric chromosomes at the centromere.

**roentgen (R)** A unit of exposure to ionizing radiation named after Wilhelm Röntgen, German scientist who discovered x-rays in 1895. It is that amount of  $\gamma$ - or x-rays required to produce ions carrying one electrostatic unit of electric charge (either positive or negative) in  $1\text{ cm}^3$  of dry air under standard conditions.

**roentgen equivalent man** See rera.

**rRNA** The RNA molecules that are the structural components of the ribosomal subunits. In prokaryotes these are the 16S, 23S, and 5S molecules; and in eukaryotes, they are the 18S, 28S, and 5S molecules.

*Saccharomyces cerevisiae* Brewer's yeast.

**sarcoma** A type of cancer derived from connective bone or fat tissues. Examples include fibrosarcoma, osteogenic sarcoma, and liposarcoma.

**satellite DNA** DNA that forms a minor band if genomic DNA is centrifuged in a cesium salt gradient. This DNA usually consists of a short sequence repeated many times in the genome.

**scan** A diagnostic test usually involving the movement or scanning of a detector to produce a picture. Examples include ultrasound, nuclear medicine, computer-assisted tomographic, and magnetic resonance scans.

**scattered radiation** Radiation that, during passage through matter, is changed in direction (the change is usually accompanied by a decrease in energy).

**SCE** Sister chromatid exchange.

**secondary cancer** Cancer arising from a primary cancer; metastatic cancer.

**segregation** The separation of homologous chromosomes into different gametes during meiosis.

**selectable marker** A gene whose expression makes it possible to identify cells that have been transformed or transfected with a vector containing the marker gene. It is usually a gene for resistance to an antibiotic.

**selection** The force that brings about changes in the frequency of alleles and genotypes in populations through differential reproduction.

**semiconservative replication** A model of DNA replication in which a double-stranded molecule replicates in such a way that the daughter molecules are composed of one parental (old) and one newly synthesized strand.

**sensitizing agent** A substance that increases the biologic effectiveness of a given dose of radiation.

**sex chromosome** A chromosome, such as the X or Y in humans, which is involved in sex determination.

**sex linkage** The pattern of inheritance resulting from genes located on the X chromosome.

**sex-linked** Genes located on the X or Y chromosome.

**sexual reproduction** Reproduction through the fusion of gametes, which are the haploid products of meiosis.

**SF<sub>2</sub>** Surviving fraction at 2 Gy.

**sickle-cell anemia** A genetic disease in humans caused by an autosomal recessive gene, usually fatal in the homozygous condition. Caused by an alteration in the amino acid sequence of the beta chain of globin.

**side effects** Symptoms directly related to treatment, such as the side effect of nausea resulting from radiation treatment over the stomach. Side effects are considered acute if they occur during treatment and subside if treatment is complete. Those symptoms persisting over a longer period of time are considered chronic.

**sievert (Sv)** Unit of equivalent dose or effective dose. One sievert is 100 rem.

**SINES** Short interspersed repetitive sequences found in the genomes of higher organisms, such as the 300-bp Alu sequence.

**sister chromatid exchange (SCE)** A crossing-over event that can occur in meiotic and mitotic cells; involves the reciprocal exchange of chromosomal material between sister chromatids (joined by a common centromere). Such exchanges can be detected cytologically after BrdU incorporation into the replicating chromosomes.

**slow repair** Long-term recovery that takes place on a time scale of weeks to months.

**solid tumor** A cancer originating in an organ or tissue other than bone marrow or the lymph system.

**somatic** Pertaining to the body; to all cells except the germ cells.

**somatic cell** Any cell other than a germ cell that composes the body of an organism and that possesses a set of multiploid chromosomes.

**somatic effects of radiation** Effects of radiation limited to the exposed individual, as distinguished from genetic effects, which also affect subsequent, unexposed generations. Large radiation doses can be fatal. Smaller doses may make the individual noticeably ill, may produce temporary changes in blood-cell levels detectable only in the laboratory, or may produce no detectable effects.

**somatic mutation** A mutational event occurring in a somatic cell. In other words, such mutations are not heritable.

**SOS** A GDP-GTP exchange protein that acts on ras proteins. Binds to the SH3 domains of GRB2 through its proline-rich motifs.

**SOS response** The induction of enzymes to repair damaged DNA in *Escherichia coli*. The response involves activation of an enzyme that cleaves a repressor, activating a series of genes involved in DNA repair.

**Southern blotting** A procedure in which DNA restriction fragments are transferred from an agarose gel to a nitrocellulose filter, where the denatured DNA is then hybridized to a radioactive probe.

**spatial cooperation** The use of radiotherapy and chemotherapy to treat disease in different anatomical sites.

**species** A group of actually or potentially interbreeding individuals that is isolated reproductively from other such groups.

**specific activity** The radioactivity of a radioisotope of an element per unit weight of the element in a sample. The activity per unit mass of a pure radionuclide. The activity per unit weight of any sample of radioactive material.

**spheroid** Clump of cells grown together in tissue culture suspension.

**spindle fibers** Cytoplasmic fibrils formed during cell division that are involved with the separation of chromatids at anaphase and their movement toward opposite poles in the cell.

**split-dose (SLD) recovery** Decrease in radiation effect if a single radiation dose is split into two fractions separated by times up to a few hours (also *Elkind recovery*, or *recovery from sublethal damage*).

**spontaneous mutation** A mutation that is not induced by a mutagenic agent.

**spore** A unicellular body or cell encased in a protective coat that is produced by some bacteria, plants, and invertebrates; capable of survival in unfavorable environmental conditions; can give rise to a new individual upon germination. In plants, spores are the haploid products of meiosis.

**stable isotope** An isotope that does not undergo radioactive decay. Compare radioisotope.

**stage** The anatomic extent of a cancer. Cancer may exist in the organ of origin and extend locally, or spread to regional tissues, then to local lymph nodes, and then to distant areas as metastases.

**standard deviation** A quantitative measure of the amount of variation in a sample of measurements from a population.

**standard error** A quantitative measure of the amount of variation in a sample of measurements from a population.

**stathmokinetic method** Study of cell proliferation using agents that block cells in mitosis.

**stem cells** Cells capable of self-renewal and of differentiation to produce all the various types of specialized cells in a lineage.

**sterility** The condition of being unable to reproduce; free from contaminating microorganisms.

**sticky end** A single-stranded nucleotide sequence produced if a restriction endonuclease cleaves off center in its recognition sequence.

**stochastic effect** Effects the probability of which, rather than their severity, is a function of radiation dose without threshold. (More generally, *stochastic* means random in nature).

**strain** A group with common ancestry that has physiologic or morphologic characteristics of interest for genetic study or domestication.

**stringency** Reaction conditions, such as temperature, salt, and pH, that dictate the annealing of single-stranded DNA/DNA, DNA/RNA, and RNA/RNA hybrids. At high stringency, duplexes form only between strands with perfect one-to-one complementarity: Lower stringency allows annealing between strands with less than a perfect match between bases.

**sublethal damage** Nonlethal cellular injury that can be repaired or accumulated with further dose to become lethal.

**submetacentric chromosome** A chromosome with the centromere placed so that one arm of the chromosome is slightly longer than the other.

**supraadditivity or synergism** A biologic effect caused by a combination of effects that is greater than would be expected from the addition of the effects of the component agents.

**survival curve** Obtained by plotting the number or the percentage of organisms surviving against the dose of radiation.

**symbiont** An organism coexisting in a mutually beneficial relationship with another organism.

**syndrome** A group of signs or symptoms that occur together and characterize a disease or abnormality.

**synergism** Two or more agents reacting together to produce a result greater than the sum of the individual agents.

**systemic** Having a widespread effect on the body as a whole, rather than just on local tissue.

**Taq polymerase** A heat-stable DNA polymerase used in PCR.

**target cell** A stem cell whose death contributes to a reduction in growth or tissue function.

**target theory** (1) A theory based on the idea that death of a cell is caused by the inactivation of specific targets within the cell. (2) The idea that the shoulder on cell survival curves is a result of the number of unrepaired lesions per cell.

**targeted radiotherapy** Treatment of disseminated cancer by means of drugs that localize in tumors and carry therapeutic amounts of radioactivity.

**TBI** Total-body irradiation.

**telocentric chromosome** A chromosome in which the centromere is located at the end of the chromosome.

**telomerase** A reverse transcriptase which polymerizes TTAGGG repeats to offset the degradation of chromosome ends that occurs with successive cell divisions.

**telomeres** These cap and protect the end of chromosomes. They contain long arrays of TTAGGG repeats. Each time a normal somatic cell divides, the terminal end of the telomere is lost.

**telophase** The stage of cell division in which the daughter chromosomes reach the opposite poles of the cell and re-form nuclei. Telophase ends with the completion of cytokinesis.

**temperature-sensitive mutation** A conditional mutation that produces a mutant phenotype at one temperature range and a wild-type phenotype at another temperature range.

**template** An RNA or single-stranded DNA molecule upon which a complementary nucleotide strand is synthesized.

**teratocarcinoma** Embryonal tumors that arise in the yolk sac or gonads and are able to undergo differentiation into a wide vari-

ety of cell types. These tumors are used to investigate the regulatory mechanisms underlying development.

**termination (stop) codon** Any of three mRNA sequences (UGA, UAG, UAA) that do not code for an amino acid and thus signal the end of protein synthesis.

**therapeutic gain factor (in hyperthermia)**

The ratio of the thermal enhancement ratio **in** the tumor to the thermal enhancement ratio **in** normal tissue. For high linear energy transfer radiations, the therapeutic gain factor is the ratio of the relative biologic effectiveness in the tumor to the relative biologic effectiveness in normal tissue.

**therapeutic index** Tumor response for a fixed level of normal-tissue damage.

**thermal dose** A function of temperature and heating time that is thought to relate well to biologic effect. It is defined to be the cumulative equivalent minutes at 43°C.

**thermal enhancement ratio (TER)** The ratio of radiation doses, with and without heat, to produce the same biologic effects.

**thermal neutrons** Neutrons in thermal equilibrium with their surrounding medium. Thermal neutrons are those that have been slowed down by a moderator to an average speed of about 2,200 m/s at room temperature from the much higher initial speeds that they had when expelled by fission.

**thermoluminescent dosimeter (TLD)** A dosimeter containing a crystalline solid for measuring radiation dose, plus filters (absorbers) to help characterize the types of radiation encountered. If heated, TLD crystals that have been exposed to ionizing radiation give off light proportional to the energy they received from the radiation.

**thermotolerance** The induced resistance to a second heat exposure by prior heating.

**thorium series** Radioactive-decay chain starting **with** thorium-232; one member of the chain is radon-220. This chain is of much less significance than the uranium-decay chain containing radon-222.

**threshold** A level (for example, of radiation dose) below which there is no observable

effect; there is no threshold for induction of cancer by radiation: All levels of radiation are considered to be harmful.

**threshold dose** The minimum dose of radiation that produces a detectable biologic effect.

**thymine dimer** A pair of adjacent thymine bases in a single polynucleotide strand between which chemical bonds have formed. This lesion, usually the result of damage caused by exposure to ultraviolet light, inhibits DNA replication unless repaired by the appropriate enzyme system.

**time—dose relationships** The dependence of isoeffective radiation dose on the duration (and number of fractions) in radiotherapy.

**tolerance** The maximum radiation dose or intensity of fractionated radiotherapy that the therapist judges to be acceptable. Usually expressed in dose units. Actual values depend on fractionation, field size, concomitant treatments, etc.

**topoisomerase** A class of enzymes that converts DNA from one topologic form to another. During DNA replication, these enzymes facilitate the unwinding of the double-helical structure of DNA.

The ability of a cell or embryo part to give rise to all adult structures. This capacity usually is restricted progressively during development.

**trait** Any detectable phenotypic variation of a particular inherited character.

**transcription** Transfer of genetic information from DNA by the synthesis of an RNA molecule copied from a DNA template.

**transfection** The uptake and expression of foreign DNA by cultured eukaryotic cells.

**transformation** In higher eukaryotes, the conversion of cultured cells to a malignant phenotype. In prokaryotes, the natural or induced uptake and expression of a foreign DNA sequence.

**transforming growth factor (TGF)** a Functional and structural analogue of epidermal growth factor. Induces the growth of epithelial cells, as well as fibroblasts and keratinocytes. May be **involved** in tumor-associated neovascularization.

**transforming growth factor (TGF)** (3)

Family of peptides that cause growth stimulation of mesenchymal cell types and inhibition of epithelial and other cell types. Induces p15 and p27 in epithelial cells, resulting in inhibition of progression through Gi.

**transient hypoxia** Low oxygen concentrations associated with the transient closing and opening of blood vessels. Sometimes called *acute* or *cyclic hypoxia*.**translation** The process of converting the genetic information of mRNA on ribosomes into polypeptides.**translocation** A break in at least two chromosomes with exchange of material; in a reciprocal translocation, there is no obvious loss of chromosomal material.**trisomy** The condition in which a cell or organism possesses two copies of each chromosome, except **for** one, which is present in three copies. The general form for trisomy is therefore  $2n + 1$ .**tritium ( $^3H$  or T)** A radioactive isotope of hydrogen with two neutrons and one proton in the nucleus. It is manmade and is heavier than deuterium (heavy hydrogen). Tritium is used in industrial thickness gauges and as a label in chemical and biologic experiments. Its nucleus is a triton.**tRNA (transfer RNA)** A small ribonucleic acid molecule that contains a three-base segment (anticodon) that, recognizes a codon in mRNA, a binding site for a specific amino acid, and recognition sites for interaction with the ribosome and the enzyme that links it to its specific amino acid.**tumor** An abnormal growth of cells or tissues. Tumors may be benign (noncancerous) or malignant (cancerous).**tumor bed effect (TBE)** Slower rate of tumor growth after irradiation resulting from **stromal** injury in the irradiated "vascular bed."**tumor cord** Sleeve of viable tumor growing around a blood capillary.**tumor necrosis factor (TNF)** Two genes, TNF<sub>α</sub> and TNF<sub>β</sub>, involved in immune-response control and inflammation. Induced

by cytokines, ultraviolet radiations, and other agents.

**uranium** (U) A radioactive element with atomic number 92 and, as found in natural ores, an average atomic weight of approximately 238. The two principal natural isotopes are  $^{233}U$  (0.7% of natural uranium), which is fissionable, and  $^{238}U$  (99.3% of natural uranium), which is fertile. Natural uranium also includes a minute amount of  $^{234}U$ .**uranium series (sequence)** The series of nuclides resulting from the radioactive decay of the uranium isotope  $^{238}U$ . The end product of the series is the lead isotope  $^{206}Pb$ . The series includes radium and radon.**variance** A statistical measure of the variation of values from a central value, calculated as the square of the standard deviation.**vector** An autonomously replicating DNA molecule into which foreign DNA fragments are inserted and then propagated in a host cell.**viability** The measure of the number of individuals in a given phenotypic class that survive, relative to another class (usually wild-type).**virulent phage** A bacteriophage that infects and lyses the host bacterial cell.**volume-doubling time** Time taken for a tumor to double in volume.**volume effect** Dependence of radiation damage to normal tissues on the volume of tissue irradiated.**waste, radioactive** Equipment and materials (from nuclear operations) that are radioactive and have no further use. Wastes are generally classified as high-level: radioactivity concentrations of hundreds to thousands of curies per gallon or cubic foot; low-level: in the range of 1  $\mu Ci$  (microcurie) per gallon or cubic foot; or intermediate: between these extremes.**wavelength** Distance between similar points on a sine wave; length of one cycle.**white blood cells** The blood cells that fight infection.**whole-body counter** A device used to identify and measure the radioactivity in the body (body burden) of humans and animals.

It uses heavy shielding (to keep out background radiation), ultrasensitive scintillation detectors, and electronic equipment.

**wild-type** The most commonly observed phenotype or genotype, designated as the norm or standard.

**X chromosome** The female sex chromosome.

**X-linkage** See **sex-linkage**.

**xenografts** Transplants between species; usually applied to the transplantation of human tumors into immune-deficient mice and rats.

**xerostomia** Dryness of the mouth caused by malfunctioning salivary glands.

**x-ray** A penetrating form of electromagnetic radiation emitted either if the inner orbital electrons of an excited atom return to their normal state (these are characteris-

tic x-rays) or if a metal target is bombarded with high-speed electrons. X-rays are always nonnuclear in origin.

**x-ray crystallography** A technique to determine the three-dimensional structure of molecules through diffraction patterns produced by x-ray scattering by crystals of the molecule under study.

**Y chromosome** Sex chromosome in species in which the male is heterogametic (XY).

**yeast artificial chromosome (YAC)** A vector used to clone DNA fragments of up to 400,000 base pairs, which contains the minimum chromosomal sequences needed to replicate in yeast.

**Z** The symbol for atomic number; the number of protons in the nucleus.

**zygote** The diploid cell produced by the fusion of haploid gametic nuclei.

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